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THE FERTILIZATION OF ALBUGO CANDIDA.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XIX.

BRADLEY MOORE DAVIS.

(WITH PLATE XXII)

THE recent studies of Mr. Stevens (**99**) upon *Albugo bliti* have yielded some exceedingly interesting results, which are very suggestive when considered and applied theoretically to a number of the Phycomycetes whose sexual processess are so little understood. Indeed, it is possible that the generally accepted views as to the homologies of sexual organs in certain members of this group may be materially changed with the newer standpoint.

It was my privilege to follow the investigation of Mr. Stevens throughout its progress. Many questions arose for future investigation to test the suggestive conditions presented by *Albugo bliti* in other Phycomycetes. It seemed also desirable to re-examine related species of Albugo, and Mr. Stevens very kindly gave me his collections of the oft-studied *A. candida*, which furnished the material for this study.

The results confirm the views of the earlier investigators, Wager (**96**) and Berlese (**98**), as to the behavior of the sexual nuclei. But a somewhat more detailed examination of a number of points in the cytology of the oogonium will, I trust, warrant the publication of this paper. The writer has also allowed

himself to discuss the subject of fertilization in which multinucleate gametes are concerned, endeavoring to present a point of view that seems to him fruitful of future results. This portion of the paper is headed Theoretical Considerations.

The material of *A. candida* came from the vicinity of Columbus, Ohio, and had been fixed by three methods, in chrom-acetic acid, by the weak formula of Flemming, and in saturated solution of corrosive sublimate. That fixed in chrom-acetic acid proved very much the best for the present study, which desired the protoplasmic contents of the cells as free as possible from all inclusions such as oils and fats. It is interesting to know that the osmic acid of Flemming's fluid so preserved the oil-like material in the protoplasm as to render it insoluble in ordinary clearing agents, and very troublesome in all preparations, because the globules stained deeply and masked the protoplasmic elements. The material killed in corrosive sublimate did not present well-preserved nuclei or mitotic figures. The sections, cut 5μ thick, were stained with safranin, gentian-violet, and orange G.

The preparations confirmed in all essentials Wager's account of the development of the oogonium and antheridium.

A most interesting stage in this process is the differentiation of the so-called "receptive spot," a region of densely staining protoplasm in the oogonium at the point where the antheridium becomes applied to the structure. It is certainly true that a papilla from the oogonium is the active agent which works its way through the cellulose walls and establishes communication with the antheridium. In *Albugo bliti* the papilla actually pushes into the antheridium, becoming a swollen process in the interior of that structure. The papilla is much less pronounced in *A. candida*, but behaves in a strictly analogous fashion.

The antheridial tube begins its development and growth into the oogonium before there is the slighest trace of the oosphere, and it may have penetrated to one fifth the diameter of the structure before that cell is differentiated.

The development of the oosphere is an exceedingly interesting and complex process. Its commencement is indicated by a

gradual accumulation of the protoplasm at the center of the oogonium. The nuclei, very variable in number (Wager estimates from 70–110), are at first in the resting condition, but very soon prepare for and pass through a mitosis. They lie in a coarsely vacuolate cytoplasm.

The first stage in the differentiation of the oosphere is illustrated in *fig. 1*, which shows a slight but not conspicuous increase in the granular protoplasm at the center of the oogonium. There soon appears, however, a remarkable structure in this region of denser protoplasm. It develops form and organization, and finally becomes a very prominent element in the oogonium. Mr. Stevens found a similar body in *A. bliti*, appearing at this period in ontogeny, and believing it to be associated with important activities in the oogonium called it the "coenocentrum."

The coenocentrum of *A. candida* is very much larger than that of *A. bliti*, and has attracted the attention of several observers. Wager was the first to recognize its protoplasmic nature, which appears unquestionable through its structure, development, and reaction to stains and clearing agents. When fully differentiated the coenocentrum is a sphere of dense, deeply-staining, slightly granular protoplasm, entirely free from inclusions, and two to four times the diameter of the nuclei. It is sometimes surrounded by a zone of lightly staining protoplasm, as is shown in *fig. 2*, through which delicate radiations may be traced. The coenocentrum develops, as has been said, from an accumulation of protoplasm in the center of the oogonium (*fig. 1*). It reaches its most beautiful state of differentiation when the appearance is that presented by *fig. 2*. Later the structure contracts somewhat, rounds itself off, and becomes a very dense body with a firm outline, as is indicated in *figs. 3–6*. It is then very conspicuous, staining deeply, but is not so plainly related to the cytoplasm as in earlier stages. Certain investigators have mistaken these later conditions for accumulations of oil or similar matter.

The differentiation of the oosphere, that is the separation of the ooplasm from the surrounding periplasm, is heralded by the

appearance of the coenocentrum. The phenomenon is remarkable. It consists of an inward movement of the protoplasm toward the center of the oogonium, and a consequent floating out of the larger vacuoles to the periphery. The position of the nuclei is also affected so that they pass from the interior to variable distances between the coenocentrum and the wall of the oogonium. At a certain stage the nuclei may be found in a hollow sphere of protoplasm, at or just outside of the position where the wall of the oospore will finally be laid down. This is the very interesting and striking condition to which Mr. Stevens has applied the term "zonation."

Zonation is a very prominent stage of oogenesis in *A. candida*, although not as conspicuous as in *A. bliti*. It is similar in all important particulars, as may be seen by comparing *fig. 2* of this paper with *figs. 61, 62, 64, 65* of Mr. Stevens' article. The agreement is made more striking by the fact that in *A. candida* also the nuclei are usually in mitosis at this time. At the completion of zonation the ooplasm may be said to be differentiated from the periplasm, and only lacks the necessary female sexual nucleus to become the oosphere ready for fertilization.

At this point in oogenesis *A. candida* and *A. bliti* depart widely from one another. The oosphere of *A. candida* is uninucleate. One of the nuclei from a point near the periphery of the ooplasm slips back to the center and takes a position near the coenocentrum (*figs. 3, 4, 5*). One may find stages where such a solitary nucleus lies midway between the coenocentrum and the periplasm, but it is exceedingly difficult to determine in such cases whether the nucleus is returning to the center of the ooplasm or passing to the exterior. In the former case it would always be a resting nucleus, in the latter it might be in mitosis.

There can be no question but that the oosphere of *A. candida* is quite generally if not universally uninucleate. There are theoretical reasons why in the light of Mr. Steven's results with *A. bliti* one may expect some variation in this particular, and that *A. candida* may at least occasionally have a multinucleate

oosphere. Indeed a few of the author's preparations look suspicious, but their rarity would make the establishment of such a point a very tiresome and laborious investigation, if possible at all.

It will be remembered that in *A. bliti* (according to Stevens' paper) a large number of nuclei pass into the ooplasm when the oosphere is differentiated, and these are doubled in number by a mitosis, so that the oosphere when ready for fertilization contains about one hundred nuclei, and may be described as a compound oosphere. Fertilization is effected by the introduction of a large number of sperm nuclei (about one hundred) from the antheridial tube, and these, after becoming distributed through the ooplasm, finally fuse in pairs with the female nuclei.

It is very surprising that two species of the same genus, organisms so closely related as are *A. candida* and *A. bliti*, should present such a remarkable distinction as regards processes of fertilization. It was this that led the author to the examination here described. But *A. candida* certainly presents, as all investigators agree, the characteristics of the usual method of fertilization, when one male nucleus fuses with one female in an oosphere. If there is any variation in this particular it has not been demonstrated, and is probably very rare, although theoretically possible. It is to be hoped that some material of this or other species will be found to bridge over the gap between the conditions illustrated by *A. candida* and *A. bliti*.

Passing on for the present to the consideration of the antheridial tube, there is nothing to be said except in confirmation of previously published accounts. The tube at the time of zonation (*fig. 2*) is very near if not directly applied to the ooplasm. It rapidly elongates and increases in size until there is presented the club-shaped structure shown in *fig. 3*, containing granular protoplasm. A nucleus, the sperm, very soon slips from the antheridium and occupies a position in the dense cytoplasm near the tip of the tube, as is shown in *figs. 3* and *4*. The delicate wall around the tip of the antheridium finally dissolves, and the sperm nucleus is introduced into the ooplasm, at first surrounded

by a quantity of granular cytoplasm from the antheridium (*fig. 5*), which immediately mingles with that of the oogonium.

Although there is no proof that more than one sperm nucleus ever enters the oosphere, it is well to note that other nuclei may be present in the upper portion of the antheridial tube. Such an example is shown in *fig. 3*, where one perfectly organized nucleus occupies the neck of the tube. It would not seem strange to the writer if material were sometimes found where several nuclei occasionally or frequently occupied the tip of the antheridial tube, and were introduced into the ooplasm at the time of fertilization.

The sperm nucleus begins to move towards the center of the oosphere, and finally fuses with the female nucleus near if not actually adjacent to the coenocentrum. *Figs. 6* and *7* show the two sexual nuclei close to one another, and *fig. 8* is of a fusion nucleus.

An important change usually comes over the coenocentrum before or during the fusion of the sexual nuclei. The body so clearly differentiated at the time when the antheridial tube first enters the ooplasm becomes less and less clearly defined, and at last completely disappears.

The dissolution of the coenocentrum may be accomplished in two ways. The structure may increase in size, the outline becoming vague, until there is finally present an irregular mass of protoplasm usually surrounding the fusion nucleus. Or the coenocentrum may fragment into several portions, as is shown in *fig. 7*, and these later swell and merge into an unorganized granular protoplasmic mass.

It seems quite certain that the coenocentrum is not a permanent structure in the protoplasm. Arising as an accumulation of protoplasm at the center of the oogonium, the coenocentrum becomes most pronounced when the oosphere is organized, but finally all trace of the body is lost in the ripening oospore.

The suggestion of Swingle (**98**) that the coenocentrum is an organ of the oosphere seems less probable when we consider its temporary character. Yet it is possible that the coenocentrum

may have important functions to perform during oogenesis. The differentiation of the ooplasm and the separation of the female nucleus from all the other nuclei of the oogonium which pass into the periplasm is a remarkable phenomenon. However, there is little or no evidence furnished by morphology as to the part which the coenocentrum might play in these activities. On the other hand, there is the possibility that the structure may represent only an effect of functions concerned with the protoplasm of the oogonium as a whole. It may be the morphological expression of dynamic activities deeply seated in the protoplasm, such as might be presumed to operate when the ooplasm collects in the center of the oogonium, when the nuclei pass outward into the periplasm, and when the female nucleus is chosen to preside over the oosphere. This point of view is worth considering, although we have not yet enough data to safely advance such a theory.

The nuclei of *A. candida* are so small that the study of the mitotic figure is very difficult. Stages of division are always numerous in preparations, because both the antheridium and oogonium present a mitosis at one stage of their development, apparently affecting all of the nuclei simultaneously. The mitosis in the oogonium takes place when the ooplasm is differentiated from the periplasm; that in the antheridium before the penetrating tube has entered the ooplasm. Metaphase is a very conspicuous stage. As is shown in *fig. 10* the spindle is entirely intranuclear. The writer was not able to establish with certainty the presence of centrosomes, but the nuclear figure is very small, and proportionally a centrosome might be expected to be exceedingly minute. The chromosomes are granular, and the number can only be estimated with great difficulty, but Wager's view that there are about 12 or 16 is probably correct. They arise from a linin network that may be readily stained in the resting nucleus (*fig. 9*). The nuclear membrane persists until late anaphase, as is illustrated in *fig. 11*, where the two sets of daughter chromosomes are shown separated and massed at the poles of the elongated nucleus. The old nuclear membrane

finally dissolves, leaving the two groups of daughter chromosomes quite apart (*fig. 12*), after which each daughter nucleus proceeds to organize its new membrane. The intervening space, at first vacuolate, becomes filled with granular cytoplasm and the mitosis is finished.

There appears to be no positive evidence that the mitoses described above for the oogonium and antheridium are reducing divisions, whatever may be the speculations upon that point. Little can be said on this subject in our present very incomplete knowledge of the mitotic figures at other periods in the life history of Albugo. The study of the oospore may lead to some important results, although the writer's incomplete observations were no more promising than the examinations of Wager (**96**), Berlese (**98**), and Stevens (**99**). An investigation of this subject is much to be desired, and should include the study of the nuclear figure at some period of vegetative activity. *A. candida*, however, is not the most favorable subject for such an examination, as the nuclei are small. *A. portulacae* or *A. bliti* would be more satisfactory.

THEORETICAL CONSIDERATIONS.

The writer must confess a feeling of considerable uncertainty as to the cytological conditions and perhaps homologies of the sexual organs found in certain Phycomycetes. Much interesting material is likely to be presented through future investigations in this field, and particularly upon those forms whose gametes are well known to be multinucleate. Accordingly any review of the present situation can only offer suggestions of what may later become established by research. The subject is a very interesting one, but the difficulties of investigation are unusual, not only on account of the necessary technique, but in the nature of the material that must be studied.

It is well known that the gametes of certain fungi contained in the Mucorales are multinucleate. Several investigators have studied these structures, and the zygospore which results from their fusion, but none have given a clear account of the cytologi-

cal phenomenon exhibited in this act of fertilization. The descriptions of Léger (**95**) and Dangeard and Léger (**94**) present some interesting conditions, which, however, are very difficult to understand in the light of generally accepted theories of fertilization, and certainly are not at present thoroughly explained.

When the term gametes was first applied to the fusing cells that formed the zygospore of a mould, it was probably in the belief that these were sexual elements pure and simple, homologous with other sexual cells, such as the oospheres and sperms of the algæ, the fusing cells in forms of the Conjugatae, or the members of a copulating pair of swarm spores. The term gamete had a strict morphological significance implying general homologies, in most cases very close, in other instances more remote, or possibly only a relationship through some earlier non-sexual form of spore.

At present the gamete probably stands in the minds of most botanists as a morphological unit, with a structure essentially the same in all realms of biology. The gamete is a uninucleate sexual body, with a greater or less amount of cytoplasm which may be very much specialized, as is illustrated in the degree of differentiation shown by the egg and the sperm.

The question that must suggest itself to many is the correctness of the use of the term gamete, with its implied homologies, when designating the fusing sexual elements in a number of Phycomycetes. To state the difficulty concretely: are the multinucleate cells that fuse to form the zygospore of the Mucorales, the antheridial tubes and oospheres of the Peronsporales and Saprolegniales, homologous with the simpler sexual elements of the Monoblepharales and Chytridiales, and the gametes of the algæ? Although we know practically nothing about the cytology of these last two groups of fungi, nevertheless the studies of Thaxter (**95**) on Monoblepharis and the structure of the Chytridiales indicate much simpler conditions than appear in the Mucorales, Peronosporales, and Saprolegniales. They probably agree in all essentials with the sexual processes of the algæ.

In the Mucorales all that we can safely affirm now is the fusion of two multinucleate masses of protoplasm. The history and fate of the nuclei is certainly not clearly understood, but the indications are rather for some complex phenomenon involving many nuclei than a simple act of fertilization, concerned with only two sexual nuclei.

The Peronosporales present two well defined conditions at the time of fertilization. The phenomenon in *A. bliti* is at variance with that established for *A. candida*, and indicated by superficial examination for several other forms. In *A. candida* the sexual act is the fusion of two nuclei, thus satisfying the requirements generally understood by fertilization. In *A. bliti* there are many sexual nuclei fusing in pairs, about 100 sperm nuclei entering a compound oosphere which contains approximately 100 female nuclei.

What are the homologies between these two species? The oogonia and antheridia are certainly homologous structures, and there is no evidence that the nuclei contained within are not likewise. There seems indeed, from the cytological data at hand, good reason to believe that these nuclei are all homologous, both those that fuse and those that remain sexually inactive, either left behind in the antheridium or lying in the periplasm of the oogonium. Our inability at present to distinguish between the various mitoses in either sexual organ offers the strongest evidence of the position above taken. The difference between these two species is perhaps then only one of the number of the nuclei that are actually sexual or gametes in the strictest morphological sense. Nuclei that are not actually sexual, either in the antheridium or periplasm of the oogonium, may stand phylogenetically for a previous condition, when these organs contained a much larger number of sexual nuclei. This point of view seems to have been in the mind of Hartog (**91**).

The antheridium and oogonium of Albugo are gametangia. However remarkable is the difference between *A. candida* and *A. bliti* it is nevertheless merely a question of the number of nuclei that become sexually functional or active gametes. In

A. bliti there are many such; in *A. candida* the number becomes reduced to two.

Although the oosphere of *Albugo bliti* is in one sense a gamete, we should be careful to note the peculiarities that make such a designation loose and undesirable. In reality, this structure, which was called a compound oosphere by Mr. Stevens (**99**), is made up of many gametes (sexual nuclei) acting cooperatively in a common mass of cytoplasm. It may appear desirable to some to give a name to such a structure, one that shall express the idea of sexuality, although it may modify the usual conception of a gamete. The writer suggests the term *coenogamete* as applicable to a multinucleate mass of protoplasm, whose individual nuclei are sexual elements.

Let us think of the sexual processes of the mould in this light. The two elements that unite to form the zygospore are multinucleate. It is possible that some or many of these nuclei are sexual in function (this awaits investigation), and if this be true the fusing masses of protoplasm will illustrate admirably the writer's idea of coenogametes. The sexual nuclei are gametes in the strict sense of the word, and the cells that contain them are gametangia.

It seems to the writer that a standpoint is presented which may be of very great service in the investigations that will be necessary to clear up the peculiar difficulties of this field of research. Although the subject must be approached almost entirely theoretically and tentatively, he ventures to suggest some possibilities to await the verdict of future studies.

Is it necessary that the oospheres of the Peronosporales and Saprolegniales be regarded as homologous with similar structures in the algæ? It is conceivable that the degree of differentiation illustrated by the oosphere may have arisen entirely independently of the algæ, after the fungal ancestors of these groups had departed from the chlorophyll-bearing thallophytes.

Let us suppose a group of fungi with gametangia that discharge motile gametes which fuse in pairs in the water after the manner of the swarm spores of many algæ. Such forms might

readily enough have come from several groups of algæ. Let us again suppose such types of fungi to leave the water and live a terrestrial life as parasites or saprophytes. Their gametangia, unable to discharge the gametes into an aqueous medium, and yet required to satisfy the chemotactic influences associated with sexuality, might find the most satisfactory escape from the difficulties of their environment in fusing with one another. In such mingling of the cytoplasm of two gametangia would be presented the possibility of the fusion in pairs of many of the sexual nuclei (gametes) in a protoplasmic medium instead of water. From such conditions might have arisen a zygospore similar to that of the Mucorales.

If a method of zygospore formation should develop in the manner indicated above, what further changes might be expected with the gradual evolution and specialization of the forms? It does not seem unreasonable to suppose that the same tendencies toward the differentiation of sex would appear here as are manifested in all groups of the algæ. It might be advantageous to reduce the number of gametes (sexual nuclei) to provide the functional female ones with a special supply of cytoplasm, and thus to organize one or more oospheres inside a female gametangium. The furthest extreme of such sexual differentiation would be similar to the conditions illustrated by *Albugo candida*, with its single functional female nucleus (gamete), associated with a large supply of cytoplasm (ooplasm). We have in *Albugo bliti* an example of what might be imagined as an earlier stage in such a process of differentiation, for the number of functional sexual nuclei or gametes is large. Nevertheless, the female gametes are collected in a differentiated region of the cytoplasm, the oosphere, and this peculiarity would be a decided advance over the conditions illustrated by the moulds.

It should be noted that the oospores resulting from this differentiation of sex would bear no homologies to analogous structures among the algæ. They would furnish, on the contrary, another illustration of the well established fact that many divergent lines of thallophytes have developed, independently of one another, the oosporic method or sexual reproduction

The bearing of such possibilities as have been considered above upon the phylogeny of the Phycomycetes is of course attractive, but hardly within the range of the present discussion

It may be questioned whether the theoretical consideration of and speculation upon such difficult subjects as these we have so briefly outlined is worth while. Certainly we have few or no facts to warrant the expression of positive opinions, yet attempts to understand clearly and explain even the most obscure subjects may suggest methods of approach to others working for the same ends, and such hopes must be their justification.

SUMMARY.

Communication between the oogonium and antheridium is established by a papilla from the former structure, which works its way through the cellulose walls into the antheridium.

The differentiation of the ooplasm is associated with the appearance of an organized spherical protoplasmic body in the center of the oogonium. This structure has been named the coenocentrum (Stevens, **99**).

A conspicuous stage in oogenesis is that called zonation. Then the nuclei, usually in mitosis, lie at or near the inner boundary of the periplasm, and the coenocentrum is very prominent in the center of the ooplasm.

The oosphere is organized after the stage of zonation, when one of the nuclei from near the periphery returns to the interior of the ooplasm, and takes a position close to the coenocentrum. There is one mitosis in the oogonium, which occurs usually at or slightly before the time of zonation. There is no proof that this mitosis is a reducing division. The spindle is intranuclear, and the nuclear membrane persists until late anaphase. It was not possible to establish the presence of centrosomes, possibly because the mitotic figure is very small. The number of chromosomes is probably 12 to 16 (Wager's estimate).

The oosphere of *A. candida* is certainly generally, if not universally, uninucleate. This point was studied with especial care, in view of the conditions described by Mr. Stevens (**99**) in *A. bliti*.

The tip of the antheridial tube at the time of zonation is very near if not directly applied to the ooplasm. As it penetrates the oosphere a nucleus slips down into the swollen end whose surrounding wall is later dissolved, and the sperm nucleus is then introduced into the ooplasm surrounded by a quantity of dense cytoplasm.

The sperm nucleus approaches the female nucleus and slowly fuses with it in close proximity to the coenocentrum.

The coenocentrum begins to disorganize at about the time of the fusion of the sexual nuclei. The dissolution may be accompanied by fragmentation, but the final result is always the same, namely an irregular ill-defined region of dense protoplasm in the oospore that finally becomes indistinguishable from the surrounding cytoplasm.

The coenocentrum is not a permanent structure in the protoplasm. Arising as an accumulation of cytoplasm, it becomes most conspicuous at about the time of zonation, finally disappearing after the fusion of the sexual nuclei. The writer suggests that it may be merely the morphological expression of the remarkable activities displayed by the protoplasm, of the oogonium as a whole at the time of the differentiation of the oosphere.

THE UNIVERSITY OF CHICAGO.

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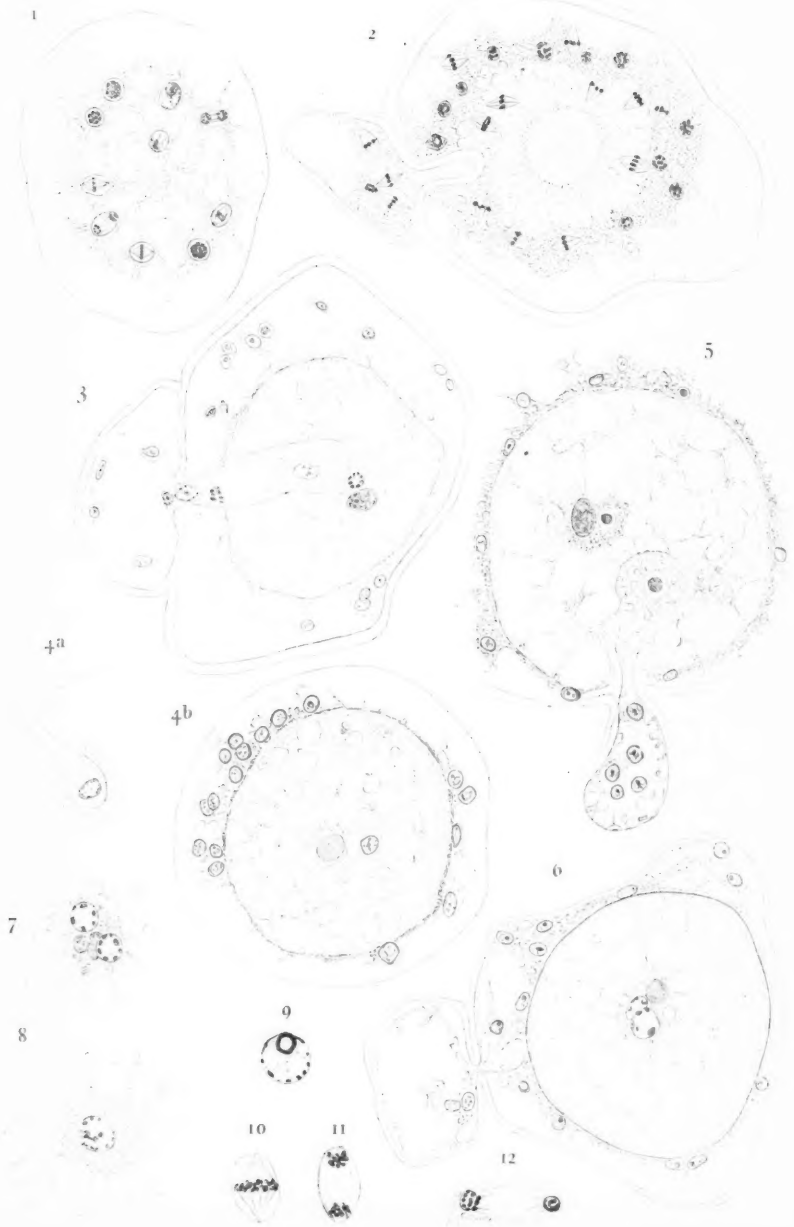


DAVIS on ALBUGO.

EXPLANATION OF PLATE XXII.

The material, fixed in chrom-acetic acid, was cut 5 μ thick and stained on the slide with safranin, gentian-violet, and orange G.

All figures sketched with an Abbé camera under the Zeiss apochromatic objective 2^{mm} ap. 1.30 in combination with compensation oculars.

Figures 1–8 magnified 1000 diameters; 9–12, 2250 diameters.

FIG. 1. Oogonium showing the first trace of the accumulation of the protoplasm in the central region preparatory to the differentiation of the ooplasm; nuclei entering or in stages of mitosis.

FIG. 2. Oogonium at the stage of zonation; nuclei mostly in metaphase and near the inner boundary of the periplasm; coenocentrum very large and surrounded by a zone of lightly staining cytoplasm; antheridial tube penetrating the periplasm.

FIG. 3. Oosphere differentiated with large deeply stained coenocentrum and smaller female nucleus; antheridial tube in the interior of the ooplasm and about ready to discharge the sperm nucleus; another nucleus in the neck of the tube, near the antheridium.

FIG. 4. Oosphere differentiated; antheridial tube (*a*) in adjacent section; coenocentrum and female nucleus shown in (*b*).

FIG. 5. Antheridium discharging its contents into the oosphere; sperm in a mass of dense cytoplasm; coenocentrum very deeply stained, with female nucleus in close proximity.

FIG. 6. Sexual nuclei fusing in oospore; coenocentrum less conspicuous than in earlier stages.

FIG. 7. Sexual nuclei approaching one another; coenocentrum fragmenting.

FIG. 8. Fusion nucleus; coenocentrum dissolved into an ill-defined region of dense cytoplasm.

FIG. 9. Resting nucleus with prominent nucleolus and delicate linin network.

FIG. 10. Nucleus in metaphase of mitosis; spindle intranuclear.

FIG. 11. Nucleus in anaphase of mitosis: nuclear membrane still persisting.

FIG. 12. Daughter nuclei after mitosis; old nuclear membrane entirely dissolved.

COMPARATIVE STUDY OF THE DEVELOPMENT OF TRICHURUS SPIRALIS AND STYSANUS STEMONITES.

H. HASSELBRING.

(WITH PLATES XXIII AND XXIV)

TRICHURUS SPIRALIS.

DURING the autumn of 1898 a hyphomycete, closely resembling the members of the genus Stysanus, was found on decaying wood brought into the laboratory of Cornell University. The principal difference between this fungus and the species of Stysanus lies in the fact that the capitulum of the former is densely beset with long, tortuous, sterile threads, recalling at first sight the spiral setae surrounding the perithecia of some species of Chaetomium. This character shows the plant to be closely related to *Trichurus cylindricus* Clements & Shear.[1] In the following pages I will refer to it as *Trichurus spiralis*, leaving a discussion of its history and nomenclature until the end of this paper. The plant was obtained in pure culture, and grown on agar and on bean stems, in order to study the development of the sporophore, and to determine, if possible, whether any other forms of fructification existed in the life cycle of the fungus.

The perfect sporophores of *Trichurus spiralis*, growing on agar or on bean stems, appear as small fluffy heads, which are either linear or elongated oblong, and obtuse or pointed at the summit. In agar they appear in small clusters of several heads near the center of the colony, but when grown on bean stems they arise over the entire surface of the substratum. Usually the sporophores are simple, but not infrequently they are branched near the base, and bear several smaller heads, or the head itself may be divided above. These rather abnormal forms are probably caused by an abundance of nutriment. While

[1] Rept. Bot. Surv. Nebraska 4:7. 1896.

fresh and growing the heads are blackish gray, but when dry they assume a copper tint, or rather a color resembling the so-called purple-brown of the dark-spored agarics. The heads are supported on thin black stalks, which are usually longer than the head itself. The entire sporophore varies in length from 0.75 to 2^{mm}; sometimes under favorable conditions larger heads are formed on bean stems, but this would scarcely occur in nature.

The microscopic structure of the sporophore is shown in *fig. 1*. The stipe is made up of many brown septate threads, growing closely together in a strict fascicle. Near their summit the threads send out many short bottle-shaped basidia, which, without being attenuated to a distinct sterigma, bear long chains of spores. Among the spore-chains there arise many long tortuous threads, which form the distinguishing characteristic of this plant. The threads are brown near the base, becoming hyaline at the tips, and give the head the characteristic flocculent appearance. From the base of the stipe numerous threads radiate in all directions. These threads are septate like those of the stipe. They are brown near their origin, but terminate in hyaline growing points. They will be more fully treated below.

The ripe spores of this plant are oval to oblong, with rounded or pointed ends (*fig. 2*). In a single preparation, however, so many variations from this form are found that this description cannot be strictly applied. Sometimes they are even and quite regular in shape; then again one or both ends may be pointed lemon-form; or the entire spore may be more or less inequilateral and irregular. Under the microscope they are dilutely yellowish-brown. The differentiation between the wall and content cannot be recognized easily, the entire spore appearing as a homogenous disk surrounded by a dark refractive ring. With high powers and careful focusing the wall and protoplasm can be made out. The spores are about 5–6 μ in length, by 2.5–3 μ in diameter.

When these spores are sown on agar, germination begins immediately, and the spore swells until it is nearly twice its

original size. At the same time the refractive ring disappears, and the protoplasm becomes distinctly granular and vacuolate. The wall on one side then seems to undergo a change. It becomes thin and delicate, until it can scarcely be distinguished, finally bulging out in the form of a short stout germ tube, whose diameter nearly equals the length of the spore (*fig. 3*). The rest of the spore membrane remains as a sort of cap over the end of the germ-tube. The entire protoplasm of the spore is used in the formation of this first germ tube. The formation of a second similar one would seem impossible, and was never observed. When this primary germ tube has attained a length about equal to the diameter of the spore, it sends out two branches from opposite sides of its broad terminal portion. This formation of a primary tube before the appearance of true hyphae is not unlike the mode of germination of the gonidia of *Completoria complens* by means of a proembryo, as described by Professor Atkinson;[2] or it may be compared to the extrusion of the endospore in *Ceratostoma brevirostre* before the formation of a true mycelium, as described and figured by Miss Nichols.[3] The first mycelial branches are thin, about 3–4 μ in diameter, and elongate rapidly, usually in opposite directions (*fig. 4*). The protoplasm in the older parts of the tubes is rather coarsely granular, with many irregular indistinct vacuoles. Near the growing portion it is homogenous, and at the tips perfectly hyaline. Numerous lateral branches soon arise in a very irregular manner from the main hyphae, being directed at a more or less acute angle to them (*fig. 5*). The branches have the same general appearance as the threads from which they arise, and equal them in diameter, and all hyphae of the mycelium contain numerous septa.

At the end of four days the mycelium has developed a well defined compact colony about 5^{mm} in diameter, with an even margin. The colony consists of a mass of threads so wedged

[2] Damping-off. Cornell Univ. Exp. Sta. Bull. 94: 233–272. *pls. 6.* 1895.

[3] The morphology and development of certain pyrenomycetous fungi. BOT. GAZ. 22: 301–328. *pls. 3.* 1896.

in together that they form a thick mat. None of the vegetative hyphae grow above the agar, so that the colony never presents a flocculent appearance.

If at this time one of the colonies is lifted out of the agar and examined under the microscope, its surface will be found to be dotted with small peculiar sporophores, resembling very much the conidial fructification of Penicillium, or still more, perhaps, on account of the slight coloration of the spores, the form genus Haplographium (*figs. 6, 7*). These gonidiophores show great variability. Those first formed consist of a single erect hypha bearing a chain of gonidia at its summit. Others have three or four branches, each with a chain of gonidia. In the older parts of the mycelium, toward the center of the colony, these small gonidiophores become more and more complex (*fig. 7*). Their branches divide and subdivide until it is impossible to represent them. In some of the gonidiophores a sterile branch takes the place of a basidium (*fig. 7*), and later, when the colony is a little older, all the gonidiophores bear long-curved setae, many times longer than the entire fruiting head, so that it sometimes seems as if a small stalk had spent all its effort in producing one of these enormous curved setae.

In these sterile threads, intermingled with the chains of gonidia, one sees a resemblance between the Penicillium form and the perfect sporophores. In time, still greater similarity appears. The heads formed near the center of the colony begin to assume a different appearance. The stalks are considerably elongated, and become brown and septate before the head of gonidia is formed (*figs. 8, 9*). Often two or three grow near together, forming a distinct stipe like that of the perfect sporophore in every respect except size. The stalk hyphae do not remain simple, but send out branches from their lower cells. These branches grow upward and soon attain a diameter equal to that of the stalk. The manner of origin of these branches can be seen best in the more simple sporophores. They are usually sent out from the lower cells, always originating immediately below a septum (*figs. 9, 10 a*). *Fig. 8* shows a small

sporophore which originated from a single erect hypha. The two lower cells have sent out branches, which, together with the original hypha, have formed a stalk bearing a small head of gonidia at its summit. The young branches are hyaline at the tips, but brown in the older portions. They soon become merged with the other hyphae forming the stipe.

In the more complex forms of the small gonidiophores there is seen an approach to the perfect sporophores of Trichurus. In fact, they are identical with the latter both in structure and manner of formation, differing only in the number of hyphae which go to make up the stipe and head. It is easy to form a complete series, showing all gradations from the simplest hypha bearing a single chain of gonidia to the most complex sporophore with several hundred spore-chains.

At a time varying from four to six days after sowing, the vegetative hyphae have formed a dense mat of mycelium in the medium in which they are growing. At the center of the colonies the threads become so numerous and interwoven that they form almost a stroma. From this stroma-like mat the perfect sporophores arise, either singly or in small clusters. They first appear as stout fascicles of hyphae, which arise perpendicular to the substratum. The growing points of the threads are hyaline, but in the older parts of the bundle they assume the characteristic brown color of the stipe (*fig. 18*, of *Stysanus stemonites*).[4] The threads branch in the manner described above, the branches remaining closely appressed to the bundle and growing with it. During longitudinal growth the central threads are in advance of the peripheral hyphae, giving the bundle a linear, pointed form. Before the bundle has completed its growth, the individual hyphae begin to send out small, curved branches or basidia at some distance from their growing points. The basidia are thus formed in centripetal order. Those first formed begin to bear chains of spores before the bundle or stipe has completed

[4] The formation of the stipe and head in the two forms, *Trichurus spiralis* and *Stysanus stemonites*, is exactly alike, and it was not considered necessary to represent this stage more than once. *Figs. 18* and *19* are from Stysanus, but they will answer equally well for Trichurus.

its growth. Finally, the threads themselves cease to elongate, their ends become rounded and enlarged, and begin bearing chains of spores like the basidia (*fig. 19*). Thus the hyphae of the stalk finally terminate in basidia. Septa are formed in the threads of the stipe somewhat later than the basidia, but their formation progresses in the same order, from the base upwards.

The basidia, as has been said, appear as short, curved, rather blunt branches. Like all growing parts they are at first hyaline, and their tips remain colorless throughout. They elongate slightly, resembling a short bottle in shape. The first spore begins to appear at the end of the basidium as a globular swelling which increases in diameter until it has reached the normal size of a mature spore. Before the first spore has completed its growth the tip of the basidium just beneath it enlarges, and a second spore appears below the first. The tip of the basidium continues thus to elongate and to cut off spores until a long chain is formed, or until the nutriment is exhausted. The exact number of gonidia thus formed in a chain cannot be well determined, as they are very much tangled in the head, and when mounted in water they break apart instantly. Sometimes, however, a gonidiophore which has sunk down into the agar is found with some of its chains partially unbroken. In these chains fifteen to eighteen spores were often counted, but the total number is probably much greater. The individual spores are connected by a short isthmus which can be distinguished with difficulty, in the mature chains, but when spores lying on the agar have begun to germinate, it can be distinctly made out as a short tube connecting the swollen spores.

The sterile threads push out from the heads soon after the formation of gonidia has begun. When first seen they are straight, being directed at right angles to the long axis of the head. They are at first hyaline, but as they grow longer they become brown. As the setae grow they twist about in various directions, but never become branched. Septa are formed at short intervals in their older parts.

In describing the perfect sporophore of this plant attention

was called to the threads which radiate evenly from the base of the stipe (*fig. 1*). From the position of these hyphae near the surface of the nutrient medium, and from the fact that they generally terminate in delicate growing points, and are not much interwoven with the threads of the mycelium, it seems that they do not originate from the mycelium, but grow out from the base of the stipe. A study of their origin showed this to be the case. Some of the branches sent out from the hyphae of the young gonidiophore are directed downwards toward the substratum (*fig. 9, b*). These branches were found on many occasions and in all stages of development, from those which had begun to others which had grown to a considerable length. It is interesting to note that those branches destined to grow upward constantly originated just below a septum, while those growing toward the substratum always began above a cross-wall. In many cases observed this was invariably true. Without doubt these radiating branches serve to support the sporophore for, as it often starts from a comparatively small bundle of hyphae, the large head of gonidia with its sterile threads would probably bear it down if it were not strengthened by further support.

The life cycle of this plant seems thus completed. Although many cultures were made in different media no perfect form was obtained, and indeed it seems probable that among some of the more highly developed Hyphomycetes like Trichurus the perfect form has been lost even if it ever existed.

STYSANUS STEMONITES.

Stysanus stemonites is a common form of the Phæostilbeæ[5] found growing on decaying wood and bark of all kinds, on which its small sporophores form gray patches of varying extent. On account of its great resemblance to Trichurus it was grown in a series of cultures parallel with those of the latter fungus, especially as the thought suggested itself that perhaps the sterile bristles might be merely an abnormal production of Stysanus. The cultures for this purpose were obtained from

[5] Sacc. Syl. Fung. 4: 603.

stock tubes in the laboratory. It is needless to say that throughout the cultures the differences between the two forms remained constant. The difference in the size of the two plants alone would be sufficient to remove all doubt as to their specific distinctness. In the course of the cultures, however, so many points of resemblance between the two plants were found, including a small form of fructification new to Stysanus, that it seems worth while to mention them here.

The spores of *Stysanus stemonites* are in general oblong or lemon-shaped, but, as in Trichurus, they are more or less irregular, and extremely variable in shape and size (*fig. 11*). They are usually more pointed than in Trichurus. Under the microscope the spores are dilutely olive-brown, almost hyaline, having the usual dark refractive line around the border. They measure from 6–7μ in length by 3–4μ in diameter, but vary outside of these limits.

Germination of the spores begins immediately after sowing. The process takes place exactly as was described for Trichurus, that is by the formation of a thick primary germ tube (*fig. 12*). After eighteen to nineteen hours the mycelium has reached the stage of development shown in *fig. 14*. The tubes are rather slender, rarely exceeding 3–4μ in diameter. The protoplasm is somewhat indistinctly granular and vacuolate.

The colonies formed by the mycelium are smaller than those of Trichurus of the same age. The hyphae do not show a great tendency to spread out in the agar but remain within a small compass. At the end of four days growth the colonies are scarcely more than 3–4^{mm} in diameter. The mycelium branches abundantly and irregularly, forming a dense mat whose center is raised slightly above the agar. The center is even more stroma-like than in Trichurus. The colonies soon become covered with a gray gonidial growth which extends almost to their margin. This growth consists of Penicillium-like gonidiophores resembling those described for Trichurus. In this case, however they are much more abundant, thus differing in a marked manner from Trichurus, in which they are never sufficiently numerous

to change the external appearance of the colony (*figs. 15, 16, 17*). The gonidiophores themselves, on the other hand, are very much alike in the two plants, as *figs. 15, 16* and *17* will show. In Stysanus also we can find a complete series showing all gradations between these small gonidiophores and the perfect sporophores.

The mature sporophores are small, rarely attaining a height of 0.75^{mm}. They arise either singly from the center of the colony, or several arise together from a stroma-like base. The stipe elongates rapidly, and soon begins to bear basidia and chains of gonidia which remain together in a tangled head so long as they are dry, but when placed in water they break apart instantly. Such sporophores from which most of the gonidia have fallen away are shown in *figs. 20* and *21*.

CONCLUSION.

From this study there can be no doubt of the close relationship of *Trichurus spiralis* and *Stysanus stemonites*. Not only are the perfect forms similar to each other, but they agree closely in their entire development, which may be summed up as follows. The spores germinate in a peculiar manner by means of a stout primary germ tube or proembryo. The mycelium forms a small, compact colony, from which there arises a gonidial fructification very different from the normal fructification of the plants. The normal fruit consists of a bundle of hyphae bearing basidia and chains of gonidia at the summit. All gradations between the simple sporophores and the compound heads exist. The characters which distingish these two plants from each other are the presence of tortuous sterile threads in the head of Trichurus and the difference in the size of the plants and spores. Among these low forms these characters are, perhaps, sufficient for generic separation. The plant treated in the first part of this paper has provisionally been referred to the genus *Trichurus* Clements & Shear. Trichurus was characterized by them thus: "As in Stysanus, but the capitulum densely beset with long strict bristles." The present plant, however, differs from this

description in the possession of curved setae. In order to include this plant in the genus, the generic description would have to be recast as follows: Stroma erect, cylindrical, dark colored, rigid; conidia borne in a loose, oblong head, ovoid or lemon-form, sub-hyaline; head beset with simple or branched, straight or curved, sterile threads or setae. The genus would include the two following species:

TRICHURUS CYLINDRICUS Clements & Shear. Rept. Bot. Surv. Nebraska 4; 7. 1896.

Trichurus spiralis, n. sp.—Sporophores 0.75—3^{mm} high, solitary or in blackish-gray patches: stipe simple or branched, black, composed of many brown septate threads: capitulum shorter than the stipe, cylindrical, obtuse or pointed at the apex, often divided into several smaller heads, beset with simple, brown, septate, tortuous bristles, which are hyaline at the end: gonidia catenulate, oval to oblong, with rounded or pointed ends, often inequilateral or lemon-form, dilutely yellowish-brown, 5–6μ by 2.5—3μ.

On decaying wood, raspberry canes, and on an insect pupa. The plant is easily recognized by the long brown sterile threads in the capitulum.

As was stated at the beginning of this paper, *T. spiralis* was obtained from decaying wood in the autumn of 1898. The same plant had been previously collected here by Professor Atkinson on raspberry canes. Specimens from cultures of this former collection were preserved, and although spores from the older material failed to germinate, the plant was easily identified as being the same as that here described.

BOTANICAL DEPARTMENT, CORNELL UNIVERSITY.

EXPLANATION OF PLATES XXIII AND XXIV.

PLATE XXIII. Trichurus spiralis.

FIG. 1. Perfect sporophore.

FIG. 2. Spores.

FIG. 3. Germinating spores.

FIG. 4. Same forming mycelium.

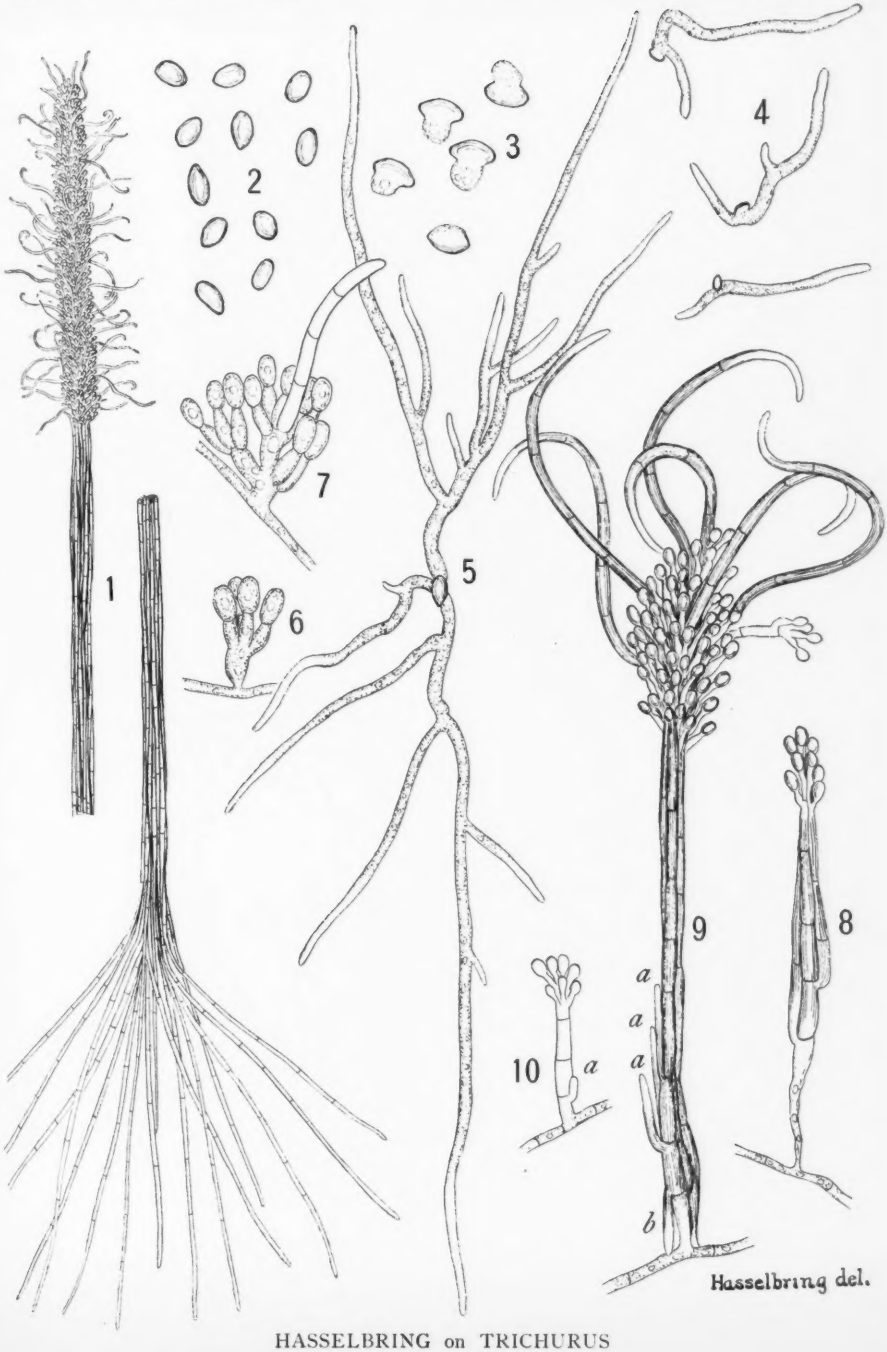
FIG. 5. Young mycelium.

FIGS. 6–7. Penicillium-fruits.

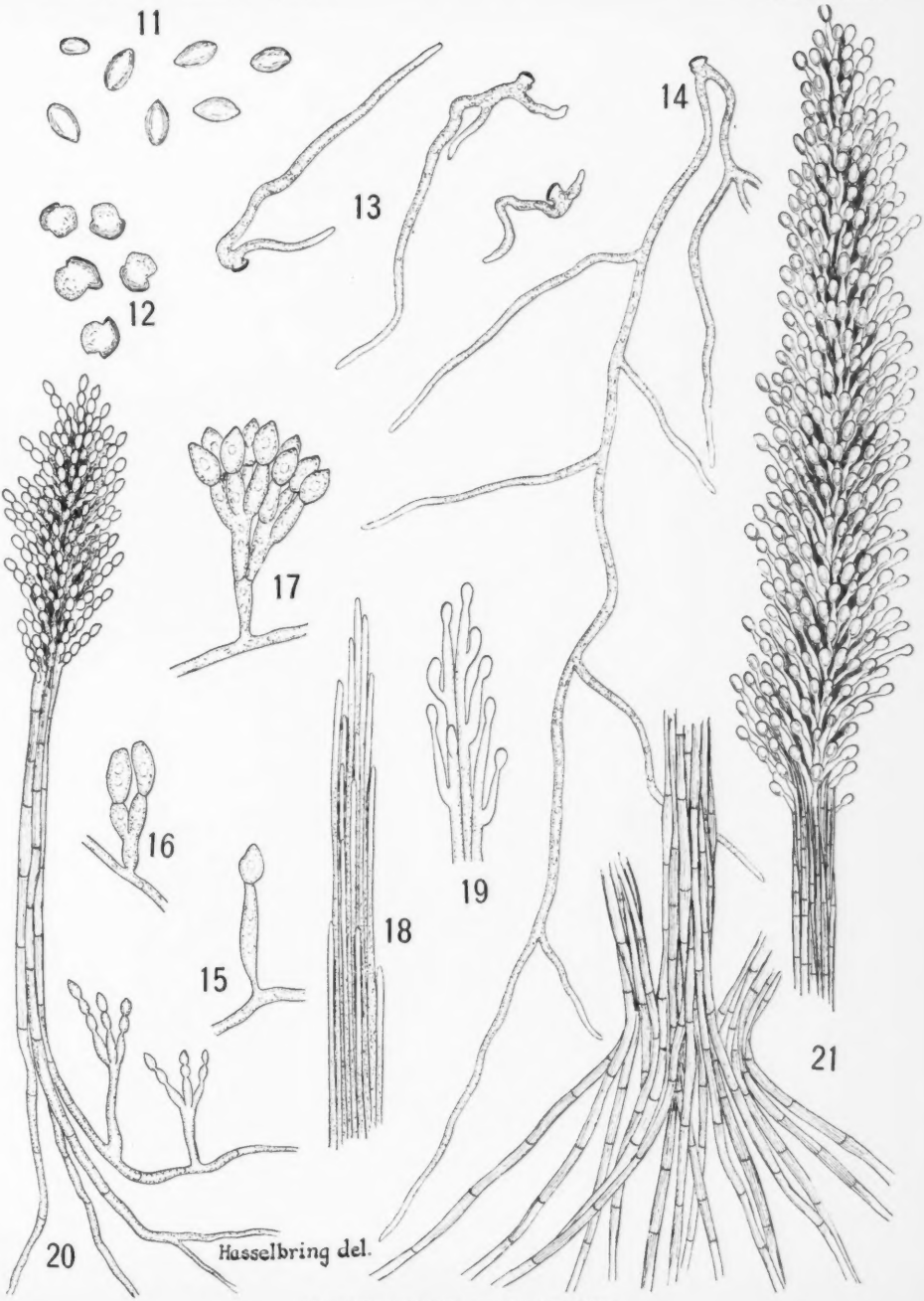
FIGS. 8–10. Small sporophores; 9*b*, descending branch; 9*a* and 10*a*, ascending branches.

PLATE XXIV. Stysanus stemonites.

FIG. 11. Spores.
FIGS. 12–13. Germinating spores.
FIG. 14. Young mycelium.
FIGS. 15–17. Penicillium-fruits.
FIG. 18. Growing sporophore.
FIG. 19. Same forming gonidia.
FIG. 20. Small perfect sporophore.
FIG. 21. Normal sporophore.



HASSELBRING on TRICHURUS



HASSELBRING on STYSANUS

THE STRUCTURE AND DEVELOPMENT OF THE SPOROPHYLLS AND SPORANGIA OF ISOETES.

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY. XVIII.

R. WILSON SMITH.

(WITH PLATES XIII–XX)

(*Concluded from page 258*)

THE SUCCESSION OF SPOROPHYLLS.

THE order of succession of the sporophylls is subject to some variation. It is not at all uncommon, especially in *I. Engelmanni*, to find the regular sequence interrupted by the occurrence of several megasporophylls among the microsporophylls. Occasionally, also, sporangia are found containing both megaspores and microspores. This is rarely the case in wild plants, though quite common, along with other irregularities, in those cultivated in the laboratory. Some plants taken in December, after growing rapidly for seven or eight months in the laboratory, had formed only megasporangia; some others, though producing a few microsporophylls, had failed to bring any microspores to perfection.

The sterile leaves of *I. echinospora* differ from the fertile ones chiefly in their smaller size, the reduction of the sheathing base, and the absence of a developed sporangium. They remain green throughout the winter; while the sporophylls, set free by decay of the base and buoyed up by the gas within the numerous air cavities, are borne away by currents or waves. A close study of the sterile leaves almost always reveals the presence of aborted sporangia. These range in size from a few to many hundred cells; they are often of irregular shape and have lost their protoplasmic contents, though now and then one is found in which a few spores have matured. A longitudinal section of a typical sterile leaf is shown in *fig. 62*, in which the shaded part

represents the undeveloped sporangium. The sterilization of the sporangium does not affect the development of the velum, a fact which supports Hofmeister's view of the primary separation of velum and sporangium. The occurrence of aborted sporangia on so many of the sterile leaves shows that all the leaves are potentially sporophylls, and suggests the probability that Isoetes has retained a more primitive form of the sporophyte than any other vascular plant.

HOMOLOGY OF THE ARCHESPORIUM.

The term "archesporium" was first employed by Goebel (1), who defined it as a cell, cell-row, or cell-plate, from which all the spore-producing cells are formed, and who concluded that in all sporangia the archesporium occupies a hypodermal position. Allusion has been made to the difficulty of accepting this conclusion in such a case as that of Isoetes, but the difficulty is not peculiar to Isoetes. Bower has shown that in several pteridophytes (Selaginella, Equisetum, Lycopodium) the archesporium is not delimited by the first periclinals of the outer layer. How shall we define the archesporium in cases where there is no single hypodermal layer from which the whole mass of sporogenous tissue is derived, and to which the term can be correctly applied as required by etymology and definition? We must either modify our conception of the archesporium or abandon the term altogether as failing to express the facts. It appears to the writer that by changing our notion of the necessary position of an archesporium we could not only avoid this difficulty but would also be enabled to make a more consistent comparison of the sporangia of seed-plants and pteridophytes than is possible with the present nomenclature.

It is pretty generally recognized that there is no true epidermis in pteridophytes. The so-called epidermis is physiologically but not morphologically equivalent to that of seed-plants, for a true epidermis is traceable to a primary layer of the embryo, the dermatogen, which is distinctly present only in seed-plants. As the dermatogen is not represented in pteridophytes, unless it

be in the root-tip, there is consequently no true epidermis, and the part which performs the functions of an epidermis is the outermost layer of the periblem derivatives. Thus, while spermatophytes have three embryonic tissue regions, the plerome, the periblem, and the dermatogen, pteridophytes, with the possible exception of the root-tip, have only the two first-named regions.

The archesporium of all spermatophytes is hypodermal. In no case is it known to be derived from the superficial cells. The epidermis is from the beginning distinct from the inner cells of the sporangium. Though there may be periclinal divisions in the superficial cells, as in gymnosperms, many Ranunculaceæ, etc., these occur only after the differentiation of the archesporium, and the cells so added merely increase the thickness of the wall or apex of the sporangium, but never become part of the sporogenous complex. The true epidermis, set apart at a very early period from the inner tissue of the embryo, is incapable of producing spore-forming cells. That rôle is played by special cells of the outer layer of the periblem.

It is otherwise with the "hypodermal archesporium" of pteridophytes. This is cut off by periclinal divisions from a superficial cell in the case of ferns and Equisetum, and from a group of such cells in the case of the Lycopodiales and Isoetes.

But if the absence of a true epidermis in pteridophytes and the homology of periblem with periblem in all vascular plants are conceded, then the hypodermal cells of spermatophytes are comparable, not to the hypodermal cells of pteridophytes, but to the superficial cells. As we have seen, the sporogenous mass in pteridophytes can always be traced to superficial cells, but in spermatophytes to hypodermal cells and no further; that is, in all cases the function of giving rise to spore-producing cells is localized in certain cells of the outer layer of the periblem.

These facts serve to show the inconsistency of undertaking to limit the archesporium to a hypodermal position in all cases. The cell or group of cells whether superficial or hypodermal, to which in a last analysis all the sporogenous portion of a sporangium can be traced, ought to be called the archesporium. The

change of nomenclature which I would propose, therefore, refers only to the position of the archesporium, and not at all to the meaning of the term. It is simply this: let the term archesporium continue to be used as at present in descriptions of seed-plants, but let it be understood in the case of pteridophytes to signify the superficial cell or cells from which the spore-forming tissue is derived. In this way the difficulties which have been pointed out will all be met, and a better system of homologies can be made for sporangia in general. The archesporium will always occupy the same position relative to the primary meristematic regions, and will be the only part from which the spore-forming tissue arises.

The nomenclature here proposed can be more easily understood by reference to the accompanying diagrams. *Figs. 69* and *70* represent two early stages of the sporangium of a common fern (Pteris), but for the present purpose may be taken as typical of any pteridophyte sporangium. The shaded cell (*a*) would be regarded as the archesporium; it divides into an inner fertile cell (*f*), from which all the spore mother cells are derived, and an outer cell (*s*) which gives rise to a large part of the sporangium wall. In some instances, as we have seen, the separation into fertile and sterile cells is not accomplished by the first division. In such cases there is no contradiction of terms, since all the spores arise from the archesporium. The final condition is the same in all cases, the difference consisting simply in the earlier or later sterilization of the wall region.

Fig. 71 represents a young microsporangium and *fig. 73* a young megasporangium of an angiosperm. The outer layer (*e*), the epidermis, takes no part in the formation of the spore producing cells; *a* is the archesporium, which usually, as in pteridophytes, divides into an outer sterile region (*s*) called the primary tapetum, and an inner fertile region (*f*) called the primary sporogenous cell or cells (*figs. 72, 74*). The name primary tapetum was given to the sterile region to express its supposed function of giving origin to the true or functional tapetum. Enough is now known of the origin of the true tapetum to enable us to say

it has no definite relation to the primary tapetum, and that in fact the term "primary tapetum" is a misnomer. The true tapetum, in many cases at least, is not represented by any morphological structure in the young sporangium.

Is it not possible that the cells *s s* of *figs. 72, 74* represent the wall layer of *fig. 70*, and that the "primary tapetum," in addition to the protective and sometimes nutritive purpose which its derivatives subserve, has also a phylogenetic meaning as a survival of the pteridophyte sporangium wall which has been in great part replaced by the true epidermis? Such at least is the view suggested by a comparison of embryonic organs in general, and of the relations of the primary sporogenous cells.

RELATIONS OF THE VELUM.

On the question whether the velum has any homologue among other plant structures my observations do not furnish any information. It has been compared on the one hand with the indusium of ferns, and on the other hand with the integument of an ovule. The possibility of the latter relation certainly has not been disproved, but the evidence for it is so scant that it must remain merely an interesting suggestion. As to the other relationship, it ought to be borne in mind that the only ferns which can be at all closely related to Isoetes are the eusporangiate families, and all of these bear naked sporangia. The indusium appears in fact to be a special organ of the higher leptosporangiate ferns, without representation in the lower families, such as the Osmundaceæ or in the eusporangiates. This absence of an indusium in the intermediate orders, and the doubtfulness of the homology of the various outgrowths known as indusia, make it impossible to regard the velum and indusium as more than homoplastic structures.

THE AFFINITIES OF ISOETES.

The systematic position of Isoetes has been discussed again and again. By Linnaeus it was placed among the vascular cryptogams, where most later taxonomists have been content to leave it. During the first half of the present century it was most frequently

grouped with the Marsiliaceæ and Salviniaceæ, chiefly on the grounds of their heterospory and hydrophytic habit. DeCandolle was the first to suggest a connection with Lycopodium. In this view he was followed by Brogniart, Endlicher, Hofmeister, and the later German botanists. A summary of the various relationships which have been assigned to Isoetes was given in 1888 by Vines (1), who in the same article put forth the opinion that its affinities are with the eusporangiate ferns, rather than with the Lycopodiales. More recently Farmer (1) and Campbell (4) have expressed their concurrence with this disposition of the genus.

Since this classification has been retained by Vines in his *Text-Book of Botany*, and adopted by Campbell in his *Mosses and Ferns*, it will not be unprofitable to re-examine the evidence, with the purpose of seeing what light can be thrown upon the subject by the present and other recent investigations.

In any discussion of relationships, and especially when there is so great diversity of opinion as in the present case, the conclusion is likely to be a personal one merely, dependent on the kind of evidence which the examiner holds most weighty, rather than on its absolute nature. There are some general principles, however, to which everyone will probably assent, and which ought to govern one in estimating the relative value of the conflicting evidence on which the taxonomist relies. In the first place, the larger the number of characters in which there is agreement, the closer is the relationship, especially if the characters are such as are known to have great taxonomic value in groups related to the one under consideration. Of single characters, those which are most constant are of most value, even though we are not able to detect their special utility. It is generally accepted, too, that those characters which appear in the embryonic stages of an organism serve best to mark its wider relationships, as of class or family, while characters which do not display themselves till later in the individual life are better adapted to distinguish the near relationships of species and genus. This principle applies not merely to the organism

as a whole, but quite as fully to the embryonic stages of its different organs, such as leaf, root, sporangium, and the like.

In conformity with these principles it is proper, in the determination of natural affinities, to place great emphasis upon the reproductive parts, for such parts are found to show very great constancy in their form and occurrence. The sporangia especially, and the form and arrangement of the sporophylls, have long been recognized as of the highest importance. Thus, the classification of the Filicales is largely based on sporangial characters; and the position of the Salviniaceæ and Marsiliaceæ, which was formerly as unsettled as that of Isoetes, was established beyond doubt as soon as the development of the sporangia was fully understood.

It is chiefly on the basis of the superficial resemblances of the sporangia of Isoetes with those of Lycopodium and Selaginella that it has been so long associated with them. If we enumerate the chief differences between the sporangia of Lycopodiales and of ferns, we shall see that in every particular Isoetes agrees with the former. While the Filicales bear numerous sporangia on the dorsal surface of the leaf, Isoetes and the Lycopodiales, with the exception of the Psilotaceæ, the exact relation of whose sporangia to the leaf is still in dispute, bear but one sporangium to a sporophyll, and that on the ventral surface at the base. Such exceptional forms as the Ophioglossaceæ and Marsiliaceæ do not help us in this inquiry. Though it may be true that the whole sporangiophore of the Ophioglossaceæ is, as several morphologists have suggested, the homologue of the single sporangium of Lycopodium or Isoetes, the suggestion is so hypothetical in itself as to give no support to any view based upon it. It is only in the position of the sporangium that these families approach Isoetes; in other sporangial characters, such as number and development, they are like other ferns.

The relative age of the leaves, when the sporangial rudiments first make their appearance, is of considerable significance. In the Filicales, with the exception of the heterosporous forms,

which being leptosporangiate cannot be closely related to Isoetes, the sporangia appear late in the history of the leaf. There is an enormous development of the midrib with its conductive tissues, and of the expanded pinnæ, before the sporangia are recognizable. Nothing is more striking, however, than the quickness with which in the Lycopodiales and Isoetes the rudiment of the sporangium follows the inception of the leaf, which when the sporangium first comes into view is no more than a mere papilla of undifferentiated tissue, without a sign of photosyntactic or conductive tissue.

Still more far-reaching is the agreement of Isoetes with the Lycopodiales in the character of the sporangium rudiment. Goebel (1) in his celebrated paper of 1880–1 classified sporangia as leptosporangiate or eusporangiate according as they arise from single cells or from groups of cells. Though the two classes are connected by transitional forms, such for instance as the Osmundaceæ, in which the sporangia, though always classified as leptosporangiate, do not arise strictly from single cells, the distinction has been approved by all later morphologists. The leptosporangiate plants make a well-defined and consistent group, but the eusporangiates comprise very diverse forms, including the several divisions of seed-plants, the Lycopodiales, the Equisetales, and part of the Filicales. If, however, we leave out accessories, and turn our attention entirely to the essential part of the sporangium, that is to the sporogenous tissue, we find a distinction which has the merit of leaving the Filicales an unbroken group, and of agreeing closely with what is required by a consideration of other characters. This distinction pertains to the origin of the archesporium. The spore-forming part of the sporangium of Isoetes and Lycopodiales can be traced back to a number of cells placed transversely to the leaf, but of all other pteridophytes to a single cell. Is not this distinction as valid as that which pertains to the origin of the whole sporangium? If so, it tends strongly to justify the inclusion of the Lycopodiales and Isoetes within a distinct group set apart from all other vascular cryptogams.

Certain other features of the sporangium of Isoetes find duplication only among members of the Lycopodiales. In all the higher leptosporangiate ferns there is an elaborate mechanism for the bursting of the sporangium and the scattering of the spores. This device, consisting of a row of peculiarly thickened cells (the annulus), and a group of cells which form an easy place of rupture (the stomium), is very rudimentary in the lower leptosporangiates (Osmundaceæ), and in the Ophioglossaceæ and Marattiaceæ, but it is not altogether absent. There is at least a predetermined line along which dehiscence shall take place. The elaboration of this dehiscence apparatus is one of the chief peculiarities of the higher leptosporangiates. When we turn to the Lycopodiales and Isoetes, however, we find positively no contrivance for dehiscence, and no vestige of an annulus or stomium. The sporangium wall is simple, and bursts by desiccation in Lycopodium and Selaginella, and by decay in Isoetes; and neither method can be regarded as a specialization.

Another analogy has been brought to light by Bower's discovery in Lepidostrobus of certain radiating strands or processes in the sporangium which are regarded by him as very probably of the nature of trabeculæ. Since the relationship of Lepidostrobus to Lycopodium can hardly be doubted, there is here a point of contact with this group of plants in a feature in which otherwise Isoetes stands alone.

Again, Selaginella and Isoetes agree very nearly in the manner of selection of the megaspore mother cells. The unselected mother cells do not divide at all, and all the spores resulting from the division of the fertile ones as a rule reach maturity. In heterosporous ferns all the mother cells divide into spores, of which but one becomes a megaspore. The contrast may be expressed in the statement that the megasporangium is differentiated in Isoetes and Selaginella *before* the tetrad division, but in heterosporous ferns not until *after* that division.

The persistence of the tapetum in Lycopodiales and Isoetes is a character to which no great importance is to be attached, for

tapetal characters are notoriously variable. Such bearing as it has, however, is in harmony with what may be inferred from other features of the sporangium. It involves no disorganization of the cells, no multiplication of nuclei except as related to cell-division, and no mingling of naked protoplasm with the young spores.

One of the facts which Vines advanced as an argument against the usually accepted classification of Isoetes is the absence of a strobilus, the characteristic arrangement of the sporophylls in the Lycopodiales. He contrasts also the elongated, slender, branched stem of Lycopodium or Selaginella with the short unbranched stem of Isoetes, which much more closely resembles that of some eusporangiate ferns. It may be doubted whether such superficial characters, unless accompanied by internal features of which they are the outward expression, have any value in settling the relationship of distinct genera or families. At all events, their usefulness in angiosperm taxonomy is limited to the distinction of species; they would be of no use in deciding the family to which an undetermined species ought to belong. I am inclined to think the whole plant-body of Isoetes can best be explained as a shortened strobilus, just such as Lycopodium would become by suppression of the stem and axis, while allowing a normal development of the leaves and sporangia.

The most obvious diagnostic character of the three groups of pteridophytes is furnished by the leaves. The leaves of the Isoetes are *sui generis*, and afford little ground for associating it with any one group rather than another. Though they are relatively few and large, as is the case among ferns, their unbranched outlines and simple tissues show an analogy with the leaves of Lycopodiales; while their peculiar vascular bundles, and chambers, and diaphragms remove them as effectually from either group. There is record, it is true, of a fossil Isoetes with a branched leaf, indicating, when taken in conjunction with the sudden reduction of the vascular bundle just above the ligule, the possibility that the present form of the leaf may be a reduced one representing a more complex ancestral type. But

we must admit, so far as mature leaf structures are concerned, that Isoetes occupies an isolated, and in no sense an intermediate position.

The testimony of the young leaves, however, is not so neutral. The form of the leaf rudiments, their manner of growth, and arrangement about the axes are the same in Isoetes as in Lycopodium and Selaginella, and quite different from what is seen among ferns. The difference is not fully expressed in saying that in one case the leaf originates from a single apical cell, and grows by means of it, and that in the other case the initiative is from a group of cells. The leaves of ferns are distinctly acrogenous, which method of growth gives them the power of assuming complex forms and allows the successive and often slow formation of stipe, pinnæ, and pinnules, and their gradual unfolding. A leaf which grows as does that of Isoetes has its power to assume a complex form limited to the time when it is meristematic throughout; as soon as the apex becomes permanent tissue the outline of the leaf is determined. The difference between such leaves is fundamental and far-reaching. A Lycopodium leaf could easily attain the size of an Isoetes leaf by retaining the meristematic power for a longer time, for they differ only in degree. The leaf of a fern could become like that of Isoetes, or *vice versa*, only by a radical change in the manner of growth.

The similarity of the leaf rudiments of Lycopodium and Isoetes is only a particular instance of a general likeness which extends to all their embryonic organs. We have already seen how this is true of the sporangia; and it holds equally good for the roots[1] and stem apex. In none of these organs is there ever an apical cell or any concentric segmentation of the apices, such as are characteristic of all the Filicales and Equisetum. A difference in this respect in the case of apical-growing organs, like the roots and stem, may not lead to important differences in the mature structures, as the variation in the stem apices of Selaginella suffices to show. But a comparative examination of

[1]Van Tieghem (1), but Bruchmann (1) entertains a different view.

meristems was shown by Bower to possess considerable phylogenetic value, in the case of ferns, and to lead to results which agree with those arrived at by a comparison of other characters. The fact that Bower has since changed his view with regard to which type of fern is more primitive does not in any way lessen the value of his previous conclusions. If we extend the series made out by him it would be in this order: typical leptosporangiate ferns, Osmundaceæ, eusporangiate ferns, Selaginella, Isoetes, and Lycopodium. In this connection the dichotomy of the roots of Isoetes, Lycopodium, and Selaginella ought not to be overlooked.

The ligules of Selaginella and Isoetes were by Goebel made the ground for grouping the two genera into one order, the Ligulatæ, though the classification was recognized by its proposer as merely one of convenience. In the former part of this paper I have made a comparison of these organs, and expressed the view that their similarity is sufficient to demonstrate their homology. If this view is correct, it furnishes additional support to the relationship of Isoetes and the Lycopodiales, especially in consideration of the discovery of a ligule in the vegetative leaves and the sporophylls[2] of Lepidostrobus, another lycopodiaceous plant.

Turning now to the gametophytes, we notice that when Vines suggested the connection of Isoetes and ferns, it was supposed that important differences existed between the female gametophytes of Isoetes and Selaginella; but the later and more complete investigations of Heinsen (1) and Arnoldi (1) have demonstrated their close resemblance. The diaphragm of the female gametophyte of Selaginella is not a true septum, and does not arise as Pfeffer (1) supposed it did, by the division of the spore into two cells. In both Isoetes and Selaginella, the free division of nuclei, their parietal placing, and the gradual extension of cell division from the periphery to the center of the spore are the same, and have no counterpart in the germination of the megaspores of heterosporous ferns. The gametophytes agree also in the absence of chlorophyll.

[2] Maslen (1).

Nearly similar evidence is furnished by the male gametophyte. Though Belajeff (1), to whom we owe the most exact investigation of the subject, says the male gametophytes of Isoetes and Selaginella afford little ground for relating the two genera, he has shown several points of resemblance, such as the separation of the prothallial (or rhizoidal) cell from the single antheridium by a cellulose wall, and the final dissolution of the non-cellulose septa of the antheridium wall, so that the spermatozoids float free in the cavity of the spore.

Though not disposed to place much dependence as a clue to the working out of phylogenetic relationships among heterosporous plants on such structures as archegonia and antheridia, which must necessarily conform more or less in shape to the space in which they are confined, I find some interest in the fact that Isoetes and Lycopodium are the only genera of pteridophytes in which the occurrence of more than two neck canal nuclei has been reported, and that in Isoetes, as in *Lycopodium Phlegmaria* and Equisetum, the plane of the division of the primary neck canal nucleus is at right angles to the archegonium axis.

The two characters which stand most in opposition to the inclusion of Isoetes in the group Lycopodiales are its multiciliate spermatozoid and the embryogeny of its sporophyte. Campbell has very properly emphasized the similarity of the Isoetes spermatozoids to those of ferns. It requires only a brief survey of the plant kingdom to show the great constancy of the form and behavior of male cells in different classes of plants. Consider, for example, the non-motile spermatozoids of the Florideæ, or the biciliate spermatozoids of bryophytes. Accordingly, if we still classify Isoetes among Lycopodiales, we must admit that the multiciliate spermatozoids make an exception to a constancy which is otherwise remarkable. Unfortunately, we have only Lycopodium and Selaginella for comparison, and are still in ignorance as to what the gametophytes of the other genera may have to tell us.

The embryo of Isoetes finds its nearest approximation in Botrychium[3] though the resemblance may be only an external

[3] Jeffrey (1).

one, due to the late differentiation of the embryonic organs and the suppression of the stem, rather than to any deep-seated likeness. However that may be, the suspensor of Lycopodium and Selaginella is a positive morphological character separating them from Isoetes. Probably the embryos of Isoetes and Botrychium can be looked upon as generalized types, the specialization taking the form in ferns of a very early demarcation of the embryonic organs, and in Lycopodium and Selaginella of a suspensor.

Two other possible reasons for relating Isoetes to ferns deserve a passing mention. Of the connection between the velum and the indusium enough has already been said; and of the agreement of the stems of Isoetes and Botrychium it is sufficent to say that the agreement is simply in the fact of secondary thickening.

The claim that Isoetes is the genus of modern pteridophytes which makes the closest approach to angiosperms, particularly to monocotyledons, gives it an interest quite out of proportion to its numerical representation. It is not clear, however, that the claim is well supported by facts. Unquestionably Isoetes and Selaginella, in their heterospory, and their intrasporic and reduced gametophytes, exhibit features of life history which run closely parallel to that of seed-plants; but such features really foreshadow monocotyledons no more than they do other seed-plants. The hypodermal archesporium, and the origin of the megaspore mother cell as the lowest of a row resulting from periclinal divisions of an archesporial cell—two points which my observations disprove — would, if established, be as strong proof of a gymnosperm as of an angiosperm attachment. Some facts distinctly favor the gymnosperm connection; these are the manner of germination of the megaspore, and the method of selection of the megaspore out of a large mass of potentially spore-producing cells, as in Cycas, Callitris, etc., to which may be added whatever favors the relationship of Isoetes with the Lycopodiales.

Professor Campbell has shown that the embryo of Isoetes bears a likeness to that of a monocotyledon in having a lateral

stem apex and a terminal cotyledon, and suggests a comparison with the embryo of Alisma for instance. The resemblance in form is undoubtedly very close, but we ought not to overlook some equally important differences. The entire absence of a suspensor in Isoetes, which has been brought forward as an objection to its close affinity with Lycopodium, militates quite as strongly against an affinity with monocotyledons; and the foot, which is particularly well developed in Isoetes, cannot be said to have any clear representative in monocotyledon embryos.

In general habit Isoetes has been compared to some grasses, rushes, and the like; this is a mere external resemblance in one of the most adaptive features of plants, and not supported by internal and essential similarities. A similar objection can be raised to the comparison of the stelar regions of Isoetes and of such monocotyledons as Dracæna. In the latter it is true there is a secondary thickening carried on by means of an extra stelar "cambium," but this cambium merely adds parenchymatous tissue within which separate vascular bundles are organized; there is nothing strictly comparable to the prismatic zone or central xylem cylinder of Isoetes. Even were the likeness much closer than it is, the peculiar stem of Dracæna, Yucca, etc., is so certainly a newly acquired, and not a primitive character, that it affords no sound reason for deriving monocotyledons through an Isoetes-like type.

To one who has followed this discussion thus far it will be evident that in the writer's opinion the balance of evidence is in favor of relating Isoetes to Lycopodium and Selaginella rather than to eusporangiate ferns. Of course the facts are not all in hand as yet, and new discoveries may materially affect the aspect of the case. The facts which the present investigations have brought to light certainly tend in the one direction. The mode of origin, development, position, and general characters of the sporangia, the development of the leaf, and the nature of the ligule point to the correctness of including Isoetes among the Lycopodiales; while the form of the spermatozoids and

embryo show the necessity of making it a separate family. The Lycopodiales so constituted comprise six genera pretty widely separated in morphological characters, as from the antiquity of the group one might naturally expect. But the extreme differences are not greater than in the Filicales. If we can include Azolla, Marsilia, the common ferns, Hymenophyllum, the Marattiaceæ, and the Ophioglossaceæ in one group, it ought not to appear inconsistent to include Psilotum, Lycopodium, Phylloglossum, Selaginella, and Isoetes in a group of coordinate rank. A fuller knowledge of the three little-known genera may tend to confirm this view, especially if they depart as widely from the remaining genera in other characters as in general habit and sporangia; but if their gametophytes, spermatozoids, and embryos agree very nearly with Lycopodium and Selaginella, it will probably be better to make of Isoetes a fourth group of pteridophytes equivalent in rank to the three now universally recognized. If an affinity with seed plants must be sought, the evidence points to a connection with gymnosperms rather than with monocotyledons.

SUMMARY.

1. The stem apex lies at the bottom of a funnel-shaped depression, around the sides of which the leaves are arranged spirally. This depression is produced by the expansion of the cortical cells of the stem in all directions.

2. The leaves arise as crescent-shaped bands of meristematic tissue. At first the basal part of the leaf (the sheath) grows most rapidly; afterwards the region of growth is transferred to the part above the ligule. There is no persistent or sharply-marked zone of meristem. The whole leaf is meristematic at first; it then gradually passes into permanent tissue, the change beginning at the apex and extending gradually downwards.

3. The air-cavities are formed out of four longitudinal bands of cells, which after losing their contents and power of multiplication are ruptured into transverse partitions by the growth of the other parts of the leaf. The size, but not the number of the air-cavities, increases with the age and growth of the leaf.

4. The ligule originates in a single vesicular cell as described by Hofmeister. The mature ligule can be distinguished into four regions: (1) the sheath which has its origin in the lowermost cells of the young ligule, (2) the glossopodium, (3) a region of living cells, and (4) a region of disintegrating cells.

5. The rudiment of the sporangium is a transverse row of superficial cells below the ligule; the upper part of this gives rise to the velum, the lower part to the sporangium proper.

6. There is no definite hypodermal archesporium. The middle cells of the sporangium *Anlage* are the first to undergo periclinal divisions. Additions to the sporogenous complex are made from the superficial cells of the sporangium.

7. The general direction of growth of the sporangium is at right angles to the face of the leaf, with a slight tendency in young sporangia to an upward direction. The cells are not in well-arranged rows or stratified layers.

8. There is no evidence that certain of the archesporial cells give rise to trabeculæ only, and certain others to mother cells only. The trabeculæ and megaspore mother cells or groups of microspore mother cells greatly outnumber the archesporial cells.

9. There is no evidence that each of the primary cells of the sporangium pursues an independent growth. On the contrary, their derivatives blend indistinguishably.

10. The microsprangia and megasporangia are indistinguishable until they have attained a volume of 15,000–25,000 cells.

11. The sporangium becomes recognizable as a microsporangium by its differentiation into irregular deeply-staining and feebly-staining radial bands. The deeply-staining regions after a period of active multiplication become the mother cells. The feebly-staining regions become the trabeculæ, walls, and tapetum,

12. The tapetum is organized out of the layer of sterile cells adjacent to the mother cells; its cells are small, densely cytoplasmic, and persistent.

13. The middle cells of the trabeculæ become elongated by compression and growth; their nuclei also become elongated and spindle-shaped.

14. The outer wall of the microsporangium is usually four layers thick, the innermost layer being part of the tapetum. The inner wall, that is the cells between the base of the sporangium and the vascular bundle, is probably formed by sterilization of cells derived from the primary cells of the sporangium.

15. The divisions of the microspore mother cells may be either successive or simultaneous. The two spindles of the second division do not become connected by secondary fibers. The microspores are usually bilateral but sometimes tetrahedral.

16. The number of microspores in a sporangium is 150,000–300,000.

17. A sporangium first becomes recognizable as a megasporangium by the marked enlargement of many or most of the cells of about the third and fourth layers. All such enlarged cells are to be regarded as potential mother cells, and the number of them which succeed in producing megaspores is probably dependent on nutrition. No tabular tapetal cells are cut off in connection with the development of the megaspore mother cells, nor is the megaspore mother cell the innermost of a row of cells formed from a single archesporial cell in a manner comparable to what is seen in the ovules of seed-plants.

18. Many cells which enlarge almost to the size of mature mother cells are finally unable to give rise to spores, but divide up into smaller cells which ultimately form part of the tapetum.

19. The trabeculæ, tapetum, and walls arise in the megasporangium as in the microsporangium, the chief difference being the greater massiveness of the single trabeculæ in the former and the much greater abundance of the tapetum.

20. No details of the division of the megaspore mother cell were obtainable. The megaspores are usually tetrahedral in arrangement, but occasionally bilateral. The number in a sporangium is 150–300.

21. The first leaves of a season are megasporophylls, and these are succeded by microsporophylls. There is occasionally some irregularity in the order of succession, and sometimes a sporangium is found which bears both kinds of spores.

22. The sterile leaves in a majority of cases have aborted sporangia. When these have made any considerable development they are usually found to show the characters of megasporangia.

23. The sporangia after all cell divisions have ceased continue to increase in volume, apparently by the osmotic properties of the substances surrounding the young spores.

24. An attempt to relate the change from megasporophylls to microsporophylls to an exhaustion of the nutritive cortical cells formed in the preceding year was unsuccessful.

25. To secure a more consistent nomenclature it is proposed to employ the term archesporium in speaking of a pteridophyte sporangium to designate the superficial cell or cells from which the sporogenous tissue takes its origin.

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McMaster University,
Toronto, Canada.

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EXPLANATION OF PLATES XIII–XX.

The drawings, except *fig. 1*, have been made with the aid of a camera lucida, and all have been reduced by photography to two fifths of their original size. Both in the text and in the explanation of the plates, the terms transverse, longitudinal, and tangential, when used to describe sections of the ligule and the sporangium are to be understood as indicating the planes in which the sporophylls were sectioned.

FIG. 1. Base of microsporophyll, inner face showing the sporangium (*s*), the velum (*v*), and the ligule (*l*). × 4.

FIG. 2. Radial longitudinal section of base of sporophyll; *s*, *v*, *l*, as in *fig.* 1. × 5.

FIG. 3. Longitudinal section of apex of a small plant. × 48.

FIG. 4. Longitudinal section of apex of a larger plant; *x*, the tracheids, *p*, the prismatic layer, *t*, the leaf traces, *m*, the cambium (semidiagrammatic). × 48.

FIG. 5. Part of the prismatic ring as seen in transverse section of the stem; *x*, *p*, *t*, *m*, as in *fig. 4*. × 160.

FIG. 6. Cortical cells from the neighborhood of the cambium. × 240.

FIG. 7. Cortical cells from the outer region of stem. × 240.

FIG. 8. Longitudinal section of a number of young sporophylls; the sporangia are indicated by the dotted outlines. × 48.

FIG. 9. Cross section of a young leaf above the ligule. × 300.

FIG. 10. Cross section of a young leaf more advanced than that shown in *fig. 9*; the position of the future air chambers is shown by the groups of nearly empty cells; the small circles of *figs. 9* and *10* indicate the side towards the axis of the plant. × 300.

FIG. 11. Part of a longitudinal section of a leaf more advanced than that of *fig. 10*, showing origin of the air cavities; *p*, the phloem, *x*, the xylem. × 300.

FIGS. 12–13. Transverse section of leaf with the vesicular cell from which the ligule originates. × 300.

FIG. 14. First division of the ligule, sectioned in plane *a*—*a* of *fig. 15*. × 490.

FIG. 15. First division of the ligule seen in radial longitudinal section. × 490.

FIGS. 16, 18. First division of terminal cell of ligule sectioned in plane *a*—*a* of *fig. 15*. × 490.

FIG. 17. The same sectioned in plane *b*—*b* of *fig. 15*. × 490.

FIG. 19. Tangential section of older ligule. × 490.

FIG. 20. Transverse section of ligule of same age as that of *fig. 19*. × 490.

FIG. 21. Transverse section of still older ligule. × 300.

FIG. 22. Median radial longitudinal section of base of half-grown ligule; *s*, the sheath, *g*, the glossopodium, *v*, the velum. × 490.

FIG. 23. Radial longitudinal section of base of mature ligule at the position indicated by *a*—*b* of *fig. 25*, showing the thickened cells of the velum and leaf adjacent to the ligule. × 48.

FIG. 24. The same in *I. Engelmanni*. × 48.

FIG. 25. Transverse section of ligule and leaf at the position indicated by *a*—*b* of *fig. 23*. × 30.

FIG. 26. Median radial longitudinal section of young sporophyll, showing rudiment of the sporangium. × 490.

FIGS. 27–28. The same more advanced. × 490.

FIG. 29. Transverse section of young sporophyll with sporangium. × 490.

FIG. 30. Transverse section of sporangium more advanced. × 490.

FIG. 31. Tangential section of young sporangium. × 490.

FIGS. 32–38. Radial longitudinal sections of young sporophyll; *fig. 37* is a section through the side of the sporangium of which *fig. 36* is a median section; *v*, the velum, *g*, the glossopodium, *s*, the sheath. × 490.

FIG. 39. Transverse section of sporangium more advanced. × 490.

FIG. 40. Transverse section of leaf with sporangium of same age as that of *fig. 39*. × 490.

FIG. 41. Median radial longitudinal section of a sporangium of about the same age as the last. × 490.

FIG. 42. Longitudinal section of side of sporangium. × 490.

FIG. 43. Transverse section of a sporangium still older but in which the trabeculæ are not yet recognizable. × 490.

FIG. 44. Transverse section of young leaves and of microsporangium at the time of the first differentiation of fertile and sterile regions; the shaded portion represents the fertile region; *v*, the velum, *l*, the ligule, *f*, the vascular bundle. × 48.

FIG. 45. Oblique nearly tangential section of microsporangium. × 48.

FIG. 46. Tangential section of microsporangium. × 30.

FIG. 47. Cross section of sporophyll and microsporangium, showing the trabeculæ, tapetum, *t*, and a few microspores; *v*, the velum (semidiagrammatic). × 30.

FIG. 48. Portion of microsporangium at the time of the first differentiation of fertile and sterile regions. × 490.

FIG. 49. The same, showing portion of outer wall. × 490.

FIG. 50. Portion of an older microsporangium, showing differentiation of sterile regions into trabeculæ and tapetum (*t*). × 490.

FIG. 51. Portion of trabecula, tapetum (*t*), and young spores of a microsporangium. × 490.

FIG. 52. Part of outer wall and tapetum (*t*) of nearly mature microsporangium. × 490.

FIGS. 53–55. Division of mother cells to form microspores; *fig. 53* illustrates successive division; *fig. 54*, simultaneous division; *fig. 55*, the shape of the spores, bilateral in *a* and *b*, tetrahedral in *c*. × 490.

FIG. 56. Young microspores. × 490.

FIG. 57. Cross section of megasporangium with young spores and tapetum (*t*). × 48.

FIG. 58. Portion of a trabecula and tapetum (*t*) of a megasporangium, × 490.

FIG. 59. Tetrahedral arrangement of young megaspores. × 490.

FIGS. 60–61. Successive division of megaspore mother cells, spores bilateral. × 490.

FIG. 62. Median radial longitudinal section of sterile leaf with aborted sporangium (shaded).

FIG. 63. Transverse section of megasporangium first distinguishable as such. × 490.

FIG. 64. Part of transverse section of a megasporangium with a group of potential mother cells. × 490.

FIG. 65. The same with a single mother cell. × 490.

FIG. 66. Part of a transverse section of a megasporangium; for explanation see text. × 490.

FIG. 67. Diagram of young megasporangium.

FIG. 68. Diagram of a longitudinal section of the stem. See p. 228.

FIGS. 69, 70. Early stages of the sporangium of a fern. Diagrammatic. *a*, archesporium; *f*, fertile sporogenous cell; *s*, sterile wall cell.

FIGS. 71, 72. Early stages of a microsporangium of an angiosperm. Diagrammatic. *a*, archesporium; *e*, epidermis; *s*, primary tapetum; *f*, primary sporogenous cell or cells.

FIGS. 73, 74. Early stages of a megasporangium of an angiosperm. Diagrammatic. Letters as in *figs. 71, 72*.

BRIEFER ARTICLES.

PHYSIOLOGICAL NOTES. I.

1. Soja beans for imbibition experiments.

No seeds, unless they have been killed, are suitable for the demonstration of imbibition except when the experiment is to run for a short time, as for a single day. But for such brief experiments — and they are as good as longer ones — the Soja beans are an especially interesting subject, because of their pronounced change of shape in swelling. The three diameters may be designated as D, the longest one, passing between the cotyledons, at a right angle with D', which also passes between the cotyledons, but through the hilum, and D'', which passes through the cotyledons. The average lengths of these in ten air-dry beans were: D, 0.30734 inch; D', 0.28061 inch; D'', 0.22583 inch. After soaking twenty-four hours in distilled water at 14° C., the measurements were: D, 0.53708 inch; D', 0.34193 inch; D'', 0.2651 inch. The increase had been: D, 74.75 per cent., D', 21.85 per cent., D'', 17.39 per cent. I have never seen a change in shape comparable to this in the swelling of any other hard and solid substances. The swelling at first is more uniform in all directions, so far that D'' is occasionally greater when imbibition is a little over half complete than when it is finished (!), though the seed coats are not wrinkled when this occurs. The increase in bulk of these beans was very nearly 150 per cent., while the increase in weight was only 133 per cent. (the dry beans are very heavy).

Working on the micellar hypothesis, we expect the power to conduct heat in any direction to vary somewhat inversely to the power to absorb water: since the greatest swelling will be in the direction of the least axes of the micellae, where the proportion of surface to mass is greatest, while heat is conducted most easily lengthwise of the micellae, in the direction of fewest breaks. Thus heat travels most readily lengthwise in wood, at a right angle to the line of greatest swelling. I tested it with these beans by the usual method, melting a very thin layer of paraffin on the surface, and sticking a hot needle in the

middle. On the smooth surfaces, where the cotyledons meet, the melted area is even in outline and easy to see. Average dimensions of ten such melted areas were: along axis D, 0.0649 inch; along D', 0.0714 inch. The diameters of the cells which conduct the heat are about equal in these two directions. The cells are elongated along the axis D'', but the cotyledons cannot be cut so as to give a surface smooth enough to work with. The result — conduction more difficult in the direction of greater swelling — harmonizes with the hypothesis. But it is hard to imagine an arrangement of the micellae by which their long axes are, for instance, parallel in the entire cellular structure, without regard to the individual cells.

2. Gas diffusion through the cuticle.

Experiments on the diffusion of carbon dioxide through the cuticle are interesting in relation to the gas nutrition of the plant, and have an even greater value as aids in the explanation of the most fundamental physical processes. For we are almost obliged to preface our treatment of osmosis with a rehearsal of the mechanical theory of gases: and then we are too apt to introduce into the particular explanation of osmosis a new element, in the semi-permeable membrane. The plant cuticle is an appropriate instance of a membrane nearly semi-permeable to gases. With the leaves usually employed the experiment is very slow, and I have tested a number of leaves in search of some one with which the course of the experiment would be enough more rapid to recommend it for class demonstration.

All the leaves in the following lot have the stomata confined to the lower surface, and are available for winter use. They were set up in the usual way: a cork fitted over one end of a glass tube, and heated in paraffin, and the leaf sealed over the hole in the middle of the cork. On each leaf all except an area of $121^{\text{sq mm}}$ over the hole was covered with paraffin. Of course it was necessary to have the upper surface of the leaf exposed, instead of placing it next the cork, as Detmer recommends for single experiments. The tubes, all 21^{cm} in length, were then filled with mercury, which was displaced in a mercury bath with carbonic acid. As only a comparison was sought, barometric readings were neglected: the temperature was reasonably constant. All were in the same mercury bath. The experiment began January 8. The height of the mercury column under the various leaves was as follows:

	Jan. 9, 1 P.M.	Jan. 10, 1 P.M.	Jan. 11. 3 P.M.	Jan. 12, 4 P.M.	Jan. 13, 3 P.M.	Jan. 15, 3 P.M.	Jan. 17, 4 P.M.	Jan. 20, 3 P.M.
Ficus (immature)	21mm	26mm	35mm	42mm	46mm	58mm	66mm	73mm
Begonia: smooth, green	18	—	30	36	36	37	43	44
Begonia: red	31	ruptured						
Hedera	27	leaked						
Cyclamen	38	61	90	110	120	140	158	184
Farfugum	34	56	70	98	118	145	157	170
"Croton" (Codiaeum)	65	123	153	168	177	184	185	184

The see-sawing, especially evident with the Cyclamen and Farfugum leaves, is a mystery. From other experiments I know that diffusion through the Hedera leaf is considerably faster than through mature leaves of Ficus. Nerium leaves are also among the more resistant. The resistance to diffusion increases with the age of the leaf and with its dryness. According to Detmer dry Hedera leaves are impermeable; but air-dry and entirely brown leaves of Ficus still permit a slow escape of CO_2. From these and a few other experiments I regard the Croton[1] leaf as the best adapted of the leaves of ordinary greenhouse plants for the demonstration within a reasonable time of the diffusion of gases through the cuticle.

What the actual process of the passage of any gas through the cuticle is would be hard to determine. Of course it is not mere diffusion, else the oxygen and nitrogen of the atmosphere would pass more rapidly than the heavier carbonic acid. It may be that they dissolve in the cuticle, the carbon dioxide much more readily: or possibly they enter into some loose chemical combination, as oxygen is taken up and given off by haemoglobin. However that is, the fact that it was under the same leaf that the mercury rose fastest, and began to fall first is probably due to its thin cuticle, so that any gas must traverse it but a short distance. And in any case the external factors governing the rate of passage would be the same.

If it is desired merely to show how fast carbon dioxide can pass through a plant membrane, without concern as to the immediate application of the experiment to ordinary plant problems, one of the water lilies is a still better subject. It should be fixed with the under surface exposed to the air. With all other conditions the same as in the other experiments, and with part of a leaf of *Nymphæa alba* as the closing membrane, the mercury rose 185 mm in four hours! In three days it passed 190 mm.

[1] The plants commonly known in greenhouses as Croton are really Codiæum.

Most physical chemists agree that so long as the semi-permeable membrane really allows none of the solute to permeate it any resistance it may offer to the passage of the solvent is without influence upon the osmotic pressure. Ostwald,[2] Speyers,[3] and Pfeffer[4] make the same statement. The argument — and it a perfect one — is, in brief, that the osmotic pressure is the aggregate impact of the molecules of the solute against a medium which they cannot permeate: this medium must contain, and hence be permeable to, the solvent, else the solute could not touch it: but as the solvent has no share in the actual pressure, it is indifferent whether its movement be free, or barely possible. Osmotic pressure, in this sense, is computed from boiling and freezing point determinations: it cannot be directly measured. *The osmotic pressure useful in plant cells*, and the osmotic pressure measured by so-called osmometers, *is not the pressure which the flying molecules of the solute exert against the restraining membrane* (or other medium), *but it is the pressure which this membrane passes along against some other, more resistant medium*, or the solvent in question which is outside it. And this practical, available osmotic pressure depends very largely upon the resistance offered by the membrane to the passage of the solvent. If several osmometers be set up with the same solution, osmosis to take place through equal areas of different membranes, it will be found after a time that the columns of liquid they support are far from equivalent. In each osmometer the height of the column measures the pressure then available for work; but it may be objected that the experiment is not yet complete.

In a plant all the osmotic pressure which does anything — whether it is to keep the plant fresh and stiff (turgescent), or to absorb water from the soil, or to transport water or food, or to fulfill an usual condition of growth — is the pressure of the protoplasm against the cell wall. If there is an increase in the osmotically active material in the cell sap, *i. e.*, in the pressure of the sap against the vacuole wall, the promptness with which there can be a corresponding increase in the

[2] OSTWALD: Solutions 103.

[3] SPEYERS: Text-book of physical chemistry 63.

[4] PFEFFER: Pflanzenphysiologie 1:120 [2 ed.]. Zur Kenntniss der Plasmahaut und der Vacuolen, etc., 302. 1890. "Die Qualität der Haut kann, so lange gelöste Stoffe nicht exosmiren, den osmotischen Druck nicht beeinflussen und meine frühere gegentheilige Annahme, die sich der allgemein verbreiteten Vorstellung anschloss ist demgemäss irrig."

pressure against the cell wall depends on how rapidly water can get into the sap, and this in turn depends, inter alia, upon the resistance the protoplasm offers to its passage; this is very slight, but still must be something. Now in practice the osmotically active matter in the sap varies constantly,[5] and therefore the osmotic pressure available for work does depend constantly upon the ease with which water traverses the semi-permeable membrane.

To return to the osmometer: the column of liquid supported rises more slowly in some cases than in others because the membrane offers greater resistance to the movement of water through it. It seems evident to me that, if the osmotic pressure is the only force at work, there will be a certain minimum of pressure below which it would not suffice to put the water in the membrane into motion at all. And in this case the height at which the column finally comes to rest will measure the total impact of the molecules against the membrane *minus* the resistance the membrane offers to the passage of the solvent. Whether the movement of the water in the membrane by its own "living force" will be more inward than outward until the entire theoretical osmotic pressure gets expression in the column supported, I do not know, but doubt it: and anyway that would be by the introduction of another source of energy. Talk is not likely to get far on a subject where there have been no experiments for twenty-three years, but it is about all that we have, pro or contra. So long though as he based his opinions on experiments, Pfeffer thought that the pressure was influenced by the resistance of the membrane. And Krabbe[6] says that at temperatures near the freezing point a state of equilibrium is reached while there is a decided tension between the periphery and axis of a cylinder of live pith submerged in water, because of the resistance to the passage of water through the outer cells.

All these considerations apply equally to the tubes closed by a membrane permeable only to the gases on one side. And the fact that the "Croton" leaf did not keep its lead does not destroy my position, because the inward diffusion of the gases of the atmosphere is a

[5] As by movement of salts or food, formation or hydrolysis of starch, etc. As I found some years ago, the turgor in the leaf of Funaria is regularly higher at night, by the pressure of 0.5 per cent. KNO_3, than it is in the morning.

[6] KRABBE, G.: Jahrb. f. wiss. Bot., **29**: 447. 1896. As the water drawn in by osmosis is held by imbibition in the wall, the experiments on imbibition are valid for osmosis. Review in BOT. GAZ. **23**: 303. 1897.

disturbing element which prevents our seeing just what would happen if the carbon dioxide were the only diffusing gas in the question; it is a case where the membrane permits some of the solute to escape.

The fact that carbon dioxide diffuses more rapidly through a cuticle if the latter is kept moist, shows that water also can pass through it, and, therefore, that in the case of a solute which cannot permeate it, the cuticle would serve as the semi-permeable membrane of an osmometer. I have set one up, using a leaf of Farfugum as the membrane, and 20 per cent. NaCl as the solution inside. It was a week before any endosmosis was apparent, and in three weeks the column rose barely 6^{mm}. I believe that though this cuticle be absolutely impermeable for the salt, such a resistance as it opposes to the water must prevent its use for the accurate measurement of osmosis.—EDWIN BINGHAM COPELAND, *University of West Virginia, Morgantown.*

A NEW SPECIES OF PUCCINIA.

Puccinia Thompsonii, n. sp.—III. Epiphyllous or occasionally amphigenous. Sori scattered, oblong to linear-oblong, 0.25–6^{mm} long, reddish to chestnut-brown, erumpent, the ruptured epidermis flanking the sides. Spores oblong-clavate, constricted at the septum; vertex rounded or sometimes obtusely pointed, thickened; base obtusely rounded; epispore rather thin, very smooth, color golden-brown or lighter, 48–68 × 15–24. Pedicel slender, hyaline, 1.5–2.5 times the length of the spore.

On *Carex stenolepis* Torr. (*C. Frankii* Kunth). Lebanon, Ind., Mar. 19, 1891, *J. C. Arthur;* Pine Lawn (St. Louis), Mo., spring of 1894, *C. H. Thompson;* Alexandria, Ind., Sept. 23, 1898, *Miss Lillian Snyder;* Greencastle, Ind., Oct. 1898, *L. M. Underwood;* Pine Lawn (St. Louis), Mo., Jan. 2, 1899, *Hume & Thompson.*

This species somewhat resembles *P. Bolleyana* Sacc., but differs from it in the more scattered, larger, oblong, lighter-colored sori and the somewhat longer and narrower spores. The epispore of the upper cell is not quite so much thickened. The species is named for C. H. Thompson, of the Missouri Botanical Garden, who first called my attention to it. In the above description the material from St. Louis is taken as the type, as its abundance has furnished ample material for study. However, the material collected at the several points in Indiana, above cited, shows no appreciable variation. I am indebted

to Dr. J. C. Arthur for kindly comparing this with other possibly allied species and for material contributed.—H. HAROLD HUME, *Lake City, Fla.*

THE TAXONOMIC VALUE OF THE STAMINATE FLOWERS OF SOME OF THE OAKS.

(WITH EIGHT FIGURES)

THE flowers of the oaks have received comparatively little attention from investigators in systematic botany. Most authors have described the flowers of a single species, usually *Quercus alba*, which they have considered as a type for the entire genus. Sargent in his *Silva* has given a brief description of the flowers of each species, but the degree of variation in the form of the lobes and the amount of pubescence is hardly touched upon.

The present study was undertaken in order to determine whether there was not a wider range of forms among the flowers, and, if so, whether this variation is constant enough to aid in the separation and limitation of the species. For this purpose the staminate flowers of the oaks in the Cayuga Flora (*Q. acuminata*, *Q. alba*, *Q. platanoides*, *Q. Prinus*, *Q. macrocarpa*, *Q. rubra*, *Q. coccinea*, and *Q. velutina*) were chosen.

Staminate flowers were collected from as many trees of each species as possible at anthesis. The flowers were opened, the stamens removed, and the perianth mounted in glycerine jelly. In order to determine the limits of the midrib it was found of assistance to warm the slide after mounting.

The oaks may be divided into two groups, one containing those which require a single season for maturing their fruit, the other requiring two seasons. Of the oaks enumerated above the first five belong to the first group, the last three to the second. This same division is substantiated by a study of the floral organs.

The first group has a six-lobed perianth that is campanulate to rotate, and has six to nine stamens. The second group is easily distinguished from the first by its closely companulate perianth, and the stamens are four or five in number. The different species in each group may be distinguished by the size and shape of the lobes, the amount of pubescence, and the presence or absence of a midrib.

QUERCUS ACUMINATA.—This species has a six-lobed, thin, and delicate rotate perianth, which is 3^{mm} in diameter. The lobes are usually

somewhat unsymmetrical, two or three being close together and separated by narrow sinuses, the others are more widely separated and the sinuses are correspondingly wider and deeper. This appearance is often due to tearing in mounting, but the natural lobing may be determined by tracing the cilia along the margin. The lobes are ovate to lanceolate and obtuse. One of the lobes is frequently somewhat broader than the others, and once or twice notched near the apex. The midrib is distinct and extends a little over one third of the length of each lobe. The perianth is pilose, and the hairs are usually tinted with red. The bract is persistent, and adheres closely to the flower when the latter is removed from the catkin. This is the only species of this region that does not lose the bract before the maturing of the flower.

FIG. 1.—*Q. acuminata.*

FIG. 2.—*Q. alba.*

QUERCUS ALBA.—The flowers of *Q. alba* are very symmetrical in outline, thus forming a strong contrast to the preceding species. The perianth is slightly campanulate, thin, delicate, and five or six-lobed. The diameter is 2–3^{mm}. The lobes are small, broadly oval to ovate, and obtuse. The midrib is prominent and distinct nearly to the apex of the lobes. The perianth is densely pilose, the hairs being long and matted, rendering it difficult to spread the flower without tearing.

QUERCUS PLATANOIDES.—The flowers of *Q. platanoides* are only about one half the size of those of *Q. alba*, being 1–2^{mm}, but resemble them closely in other respects. The texture of the perianth is thicker. It is rotate rather than campanulate, and forms a flat six-lobed disk at the base of the stamens. The lobes are broadly oval to ovate, and separated by broad and obtuse sinuses. The midrib is distinct nearly to the apex of each lobe. The perianth is pilose, but the hairs are not so thickly matted as in *Quercus alba*.

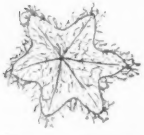

FIG. 3.—*Q. platanoides.*

FIG. 4.—*Q. Prinus.*

QUERCUS PRINUS.—The perianth of *Q. Prinus* is small, thin, and deeply lobed. The diameter is from 2–2.5^{mm}. It resembles the flowers of *Q. alba*, but the sinuses are much deeper and narrower, and the

lobes are more spatulate or obovate. There is also a variation in the number of lobes; in the majority of flowers examined there were from six to eight, but occasionally one was found with as many as ten. The midrib is distinct at the base, but disappears near the apex of the lobes. The perianth is sparingly pilose, with the lobes ciliated by long slender hairs, giving it a very shaggy appearance.

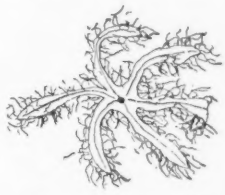

FIG. 5.—*Q. macrocarpa.*

QUERCUS MACROCARPA.—*Q. macrocarpa* has a very characteristic flower. It is more deeply lobed than any of the preceding species, the sinuses extending nearly to the point of attachment. It is the largest of this group, having a diameter of 3–3.5^{mm}. The lobes are long and very narrowly linear, five or six times as long as they are wide, and are frequently slightly curled or twisted, making it difficult to separate them from the filaments of the stamens. The midrib is distinct to the apex of the lobes. The perianth is pilose, the hairs being very long and shaggy.

FIG. 6.—*Q. rubra.*

QUERCUS RUBRA.—The flowers of *Q. rubra* are much larger than any of the other forms studied, having a diameter of 4–4.5^{mm}. They are campanulate and indistinctly five or six-lobed. As the flowers develop the stamens push through the narrow throat and tear the perianth into uneven segments varying in number from two to six. The midrib is distinct and slightly thickened at the base, but becomes indistinct about half way to the apex of the lobes. The perianth is nearly smooth or slightly pilose, and the lobes are thinly ciliate with long hairs.

FIG. 7.—*Q. coccinea.*

QUERCUS COCCINEA.—*Q. coccinea* has a perianth that very closely resembles the one just described, although the other characters of the tree are more frequently confused with *Q. velutina* than with *Q. rubra*. The perianth is campanulate and slightly six-lobed, with a diameter of 3.5–4^{mm}. Its four or five stamens as they develop tear it into two or three irregular segments. The midrib is distinct at the base but very soon disappears. The perianth is pilose, and there is a thicker row of cilia along the margin than was found in *Q. rubra*.

QUERCUS VELUTINA.—The last of this series, *Q. velutina*, has a very closely campanulate, thin and delicate perianth. The diameter is the same as in *Q. coccinea*, 3.5–4^{mm}. The midrib is entirely wanting. The perianth is pilose, and the cilia along the margin are long and matted. The throat of the perianth is so narrow that it is torn by the stamens, as soon as they begin to develop, into two or three nearly equal segments. In the majority of cases the perianth had separated into two equal segments, but occasionally one was found with three, never with more.

FIG. 8.—*Q. velutina.*

The results of this study show that there is marked variation among the flowers, not only in the case of the large groups, but also among the species in each, and the following key was based on these characters:

KEY TO NATIVE OAKS BASED ON THE STAMINATE FLOWERS.

A. *Stamens six to nine.*
- I. Bract persistent after anthesis . . . . . *Q. acuminata*
- II. Bract deciduous before anthesis.
  - *a*. Perianth deeply lobed.
    - 1. Lobes narrowly linear . . . . . *Q. macrocarpa*
    - 2. Lobes obovate or spatulate . . . . . *Q. prinus*
  - *b*. Perianth with shallow lobes.
    - 1. Perianth slightly campanulate: diameter 2–3^{mm} . *Q. alba*
    - 2. Perianth rotate: diameter 1–2^{mm} . . . *Q. platanoides*

B. *Stamens four or five.*
- I. Midrib wanting . . . . . *Q. velutina*
- II. Midrib present.
  - *a*. Perianth pilose . . . . . *Q. coccinea*
  - *b*. Perianth smooth with lobes thinly ciliate . . *Q. rubra*

—W. W. ROWLEE and SUSIE P. NICHOLS, *Cornell University*.

OPEN LETTERS.

PROFESSOR W. PFEFFER AND THE ACTIVE ALBUMIN.

THE recent publication of the second edition of Pfeffer's *Physiology of Plants* in English[1] contains on pages 67–69 the same erroneous statements and ideas in regard to the active albumin studied by Bokorny and myself as does the original German edition published two years ago: which induces me to make the following remarks.

Those who are particularly interested in the matter are referred to the protest I published two years ago under the title "Ueber Protoplasma und actives Eiweiss, zur Abwehr."[2] I have since published an extensive description of the chemically labile protein stored up as reserve material in many vegetable objects, a protein which easily changes chemically into an insoluble compound, and coagulates under the same conditions that cause the death of the protoplasm, although more slowly. This detailed account, the result of work through a long series of years, is contained in chapters nine and ten of my treatise "Die chemische Energie der lebenden Zellen."[3]

Pfeffer has wrongly interpreted or apparently overlooked certain chemical phenomena. Pfeffer's conscience seemed, however, to have dictated as a sort of excuse the following passage in the preface of his above mentioned work: "Still I am afraid that in spite of all my care much has been forgotten or overlooked, especially since, owing to the pressure of other duties, the time necessary for this work has been obtained with difficulty. This has rendered it *impossible for me to study certain problems as deeply as I had wished.*"—OSCAR LOEW, *Agricultural Department, Washington, D. C.*

[1] PFEFFER, W.: The Physiology of Plants, translated by Alfred T. Ewart. Oxford. 1900.

[2] Botanisches Centralblatt **74**: 5. 1898.

[3] München, 1899, E. Wolff, publisher. In a more condensed form it was published in London, 1896, under the title "The Energy of Living Protoplasm." Kegan Paul, Trench, Trübner & Co.

CURRENT LITERATURE.

BOOK REVIEWS.

Elementary text-books.

PROFESSOR Atkinson's *Lessons in Botany*[1] is announced as an abbreviated and simplified edition of the author's *Elementary Botany*, reviewed in this journal for December 1898. The design is to meet the requirements of those secondary schools, still far too numerous, in which not more than a half year is given to botany. The contents are clearly separated into three uses: "exercises for the pupils, demonstrations by the teacher, and descriptive matter for reading and reference." The book is more than a mere condensation of the larger one, for a large part of the matter has been rewritten, new illustrations have been introduced, and the whole presentation has a different constituency in view. As was said in the former review, Professor Atkinson is a very successful teacher and has had much experience with the schools, so that this book, as well as the other, should prove of large service in introducing into the secondary schools the truer views of botany which they so much need.—J. M. C.

AN author who writes from a university standpoint for the secondary schools has a difficult task. His own breadth of knowledge, and his relation to his fellow-workers invite to a broad, comprehensive, and up-to-date treatment of his subject. The secondary school, on the other hand, insists upon a work that is elementary in fact as well as in name.

The *Outlines of Plant Life*[2] is another expression of the desire on the part of teachers of botany to present the subject to the secondary schools in such a manner as to indicate in some measure the present condition of the science, and at the same time to give a usable book. Its appearance as an abridgement of *Plant Life* is a recognition of the fact that the present need of the secondary schools is a simpler and less comprehensive treatment of the subject. Almost a third of the original text, with the accompanying illustrations, has been cut out; some of the introductory portions have been rewritten, summaries added to each chapter, and appendix I (Directions for laboratory study) has been incorporated with the text as exercises. These changes certainly render the book much better adapted to the work of the secondary

[1] ATKINSON, GEORGE FRANCIS.—Lessons in botany. Small 8vo. pp. xv + 365, *figs. 277*. New York: Henry Holt & Co. 1900.

[2] BARNES, CHARLES REID. Outlines of plant life. Small 8vo. pp. vi + 308, *figs. 250*. New York: Henry Holt & Co. 1900.

school. The rearrangement of the laboratory work is particularly commendable. The logical arrangement, clear treatment, and freshness of the larger book are retained in this volume. There is still left a heavy year's work for a good teacher and a strong class, and it is the judgment of the reviewer that further simplification might have been secured by the less minute treatment of some subjects, as the root, movement of plants, growth, and ecology, admirable as those subjects are in their present form. The many good points of the book will commend it to secondary teachers, whose class room experience alone can determine its fitness to survive.—I. N. MITCHELL, *State Normal School, Milwaukee, Wis.*

The white pine.[3]

THE white pine has always commanded more than its share of study because of its economic importance. In so far as Professor Spalding and Dr. Fernow have succeeded in explaining the geographic and edaphic factors which control its distribution, they have made a valuable contribution to ecology and plant geography. The large number of acre-yield tables of measurements and explanations of soil and forest conditions enable them to draw certain definite and scientific conclusions regarding the causes of distribution.

The leading ecological factor is the nature of the soil and the water content of the same. While adapting itself to almost any variety of soil, the white pine prefers one with a fair admixture of sand, insuring a moderately rapid drainage. This is a soil intermediate between the stiff clayey soil on one hand, where the deciduous forest predominates, and the dry, light sandy, coarse and gravelly soil on the other hand, where the red pine (*Pinus resinosa*) and the jack pine (*Pinus Banksiana*) seem able to outdo it. The shallow root system of the white pine permits it to occupy the thinner soils of the rocky slopes in the Adirondacks and New England states.

Light is another important ecological factor. Compared with other pines the white pine has great shade endurance, hence its admixture with maples and beech, where it has an equal chance in open places. But white pine seedlings are never found in the dense shade of the hardwood forest; in such a place there is little hope for the white pine to gain a foothold. Owing to this inability of the white pine seedling to maintain itself in the dense shade, the hardwood forest is gradually encroaching upon it, except in soils too poor for the development of the deciduous forest.

A carefully prepared map of the geographic distribution offers a foundation for the discussion of the climatic factors governing such distribution.

[3] SPALDING, V. M.: The white pine. Revised and enlarged by B. E. FERNOW. Contributions on insect enemies of the white pine by F. H. CHITTENDEN, and on the wood of the white pine by FILIBERT ROTH. Bulletin no. 22. U. S. Department of Agriculture, Division of Forestry. 4to. pp. 185. *pl.* —. 1899.

It is pointed out that the white pine is delimited by conditions of humid and cold atmospheres such as are found in northern latitudes and high altitudes. Its distribution is perhaps more dependent on humidity than on temperature, or rather on a low transpiration factor, that is, such a relation of heat and moisture, both at the foot and at the top, that the thin foliage can readily perform its function.

Besides the distribution of the white pine, the monograph treats of the white pine lumber industry, the original stand and present supplies, the morphological characters, the seed and seed supply, the structure and development of the wood, the growth and development in open stand and in the forest, growth in volume, the yield of white pine, and dangers and diseases. Contributions by two specialists on insect enemies and wood of the white pine add to the economic interest. The appendix, which comprises more than half the monograph, consists of seven sets of tables of measurements, and a careful explanation of the forms used in the investigations.—H. N. WHITFORD.

MINOR NOTICES.

AMONG THOSE who have taken advantage of the offer of the Division of Forestry to make a personal study of areas which offer favorable opportunities to illustrate forest management are the owners of two large tracts of land in the Adirondacks. The results of the investigation are embodied in a recent bulletin[4] of the Department. The author makes a plea for an American system of forestry. He discusses six measures of prime importance for the proper care of the European forest, though they would be exceedingly impracticable if applied to the plots under consideration, and then proceeds to propose a simple system of management that will enable the owner to make a profit from the land and at the same time secure the permanence of the forest.

Among the features of great interest to the ecologist is the special consideration of the spruce. Under this head the habits of the spruce, the influence of situation and soil on its character and distribution, its tolerance of shade, and its reproduction are discussed in a clear and scientific manner. Another valuable feature is the presence of a large number of illustrations, mainly from photographs, and of two contour maps of the regions described. —H. N. WHITFORD.

DR. C. F. MILLSPAUGH has issued as Vol. II, no. 1, of the Botanical Series of the Field Columbian Museum the first part of his *Plantae Utowanae*, being a catalogue of the species. The plants were collected between December 1898 and March 1899 in Bermuda, Puerto Rico, St. Thomas, Culebras, Santo Domingo, Jamaica, Cuba, The Caymans, Cozumel, Yucatan, and the

[4] GRAVES, HENRY S.: Practical forestry in the Adirondacks. Bulletin no. 26, U. S. Department of Agriculture, Division of Forestry.

Alacran shoals, during the Antillean cruise of the yacht Utowana. An itinerary gives information as to the time devoted to each station, and a second part will contain a detailed account of the ecological observations, and also the plates made from the many fine photographs secured. The list of species is surprisingly long when one considers the shortness of the time and the often unfavorable conditions for collecting. Thirty-four new species are described, distributed from the fungi to the Compositae.—J. M. C.

MR. E. W. HERVEY, of New Bedford, Mass., has published independently his observations on "The colors of flowers."[5] In it he records a large amount of work which might have been better directed had he made himself familiar with the rather extensive literature on the subject. In a section on the sequence of color he assails with a vigor and directness worthy of a better object the shallow generalizations of Grant Allen on this subject. His presentation has no logical sequence and no definite marshaling of the many discrete facts which he has gathered respecting the relation of insects to color, nor any adequate correlation of these facts with the host of previous observations.—C. R. B.

THE MOST recent fascicles of Engler and Prantl's *Natürlichen Pflanzenfamilien* are nos. 193 and 194. The former contains the conclusion of Plectobasidiineæ (Sclerodermineæ) by ED. FISCHER, and the beginning of *Fungi imperfecti* by G. LINDAU. The Sphæropsidales are taken up, the 107 genera of Sphærioidaceæ being presented, and the Nectrioidaceæ begun. No. 194 contains the conclusion of Polypodiaceæ, Parkeriaceæ, Matoniaceæ, Gleicheniaceæ, Schizæaceæ, and Osmundaceæ, all by L. DIELS, and the beginning of Hydropteridineæ by R. SADEBECK.—J. M. C.

NOTES FOR STUDENTS.

SOME INTERESTING NOTES on *Arceuthobium pusillum* have been published by Hermann von Schrenk in *Rhodora* for January.

PROFESSOR J. M. HOLZINGER has found *Grimmia teretinervis* Limpr. growing near Winona, Minn. The species is new to North America.

AN ANNOTATED LIST of the puff-balls, slime-moulds, and cup-fungi of Orleans county, New York, has been published by Dr. Charles E. Fairman, of Lyndonville, in Proc. Rochester Acad. Sci. 3: 206–220. 1900.

DR. ROLAND THAXTER continues to discover new Laboulbeniaceæ. In a recent paper he describes 68 new species and 7 new genera of this family. This great extension of our knowledge of these fungi is in part the result of an examination of insect collections in the British and Paris Museums from all over the world, and of material from correspondents, as well as that gathered by the author in Maine, Florida, and elsewhere.—C. R. B.

[5] 8vo. paper, pp. 105, *figs.* 3.

MM. RENAULD and CARDOT describe in the ninth fascicle of their *Musci exotici novi vel minus cogniti*[6] a large number of species of mosses of Costa Rica and Mexico.—C. R. B.

DR. A. W. EVANS has published[7] a monograph on the Hawaiian Hepaticae of Schiffner's tribe Jubuloideæ, with full descriptions and sixteen admirable plates. Eight new species are described. The collections of Mr. C. M. Cooke, Jr., made in the summers of 1897, 1898, and 1899 mainly on Oahu and Kauai have furnished a large amount of interesting material.—C. R. B.

THE farmer's bulletin no. 112,[8] on *Bread and the principles of bread making*, prepared by Helen M. Atwater, is botanical in so far as it deals with yeasts and the structure of grains. Both these topics are treated most inaccurately. Indeed the whole bulletin strikes one as an inexpert compilation which experienced bread makers will laugh at and the inexperienced consult in vain for help.—C. R. B.

MR. R. G. LEAVITT has published[9] the results of some experiments to determine whether or not the roots of orchids and the shoots of Tillandsia, Sphagnum, and Leucobryum could condense water vapor from moist air. His results confirm those of Nabokich, published shortly before,[10] and it now seems highly improbable that the structures like the velamen of orchid roots and the hyaline cells of sphagnum are adapted to secure water in the form of vapor.—C. R. B.

A PECULIAR embryo-sac in *Peperomia pellucida* is reported by D. H. Campbell.[11] The megaspore originates in the usual way, but after the third division of the megaspore nucleus the eight nuclei are arranged about the periphery of the sac without any suggestion of polarity or differentiation in egg-apparatus, antipodals, and polar nuclei. The most striking fact is that still another division takes place, giving rise to sixteen nuclei, which are also arranged about the periphery of the sac. One of the nuclei in the micropylar end of the sac enlarges somewhat, and is to be regarded as the egg nucleus, but there are no distinct synergids, and no antipodals, although there is some grouping of a variable number of nuclei in the chalazal end of the sac. In one case the male nucleus, which is small and somewhat spirally coiled, was observed within the egg. The first division of the embryo, which has no

[6] Bull. Soc. Roy. Bot. Belg. **38**: 1–48. (Paged 1–48, and bound in second fascicle of vol. 38, but not continuous with remainder of fascicle.)

[7] Trans. Conn. Acad. Sci. **10**: 387–462. *pls. 44–59*. 1900.

[8] U. S. Dept. of Agriculture.

[9] Rhodora **2**: 29, 63. 1900.

[10] Résumé in BOT. GAZ. **29**: 222. March 1900.

[11] Ber. d. deutsch. bot. Gesell. **17**: 452–456. *pls. 31*. 1899. See also Ann. Bot. **13**: 626. 1899.

suspensor, is vertical, and the second transverse. In the ripe seed the embryo fills the entire sac, but no organs are differentiated. The free nuclei of the embryo-sac never divide, but the perisperm performs the function of endosperm. The writer regards Peperomia as a transition form between angiosperms and lower seed plants or perhaps higher pteridophytes. It is a very old type, and apparently should be placed at the beginning of the angiosperms.—CHARLES J. CHAMBERLAIN.

RECENT STUDIES on the conidia of the Entomophthorales by Cavara[12] have shown two types in the structure and development of these organs. The conidia of *Empusa muscæ* are multinucleate, for the conidiophores are characteristically cœnocytes, and the constriction of the conidium from the tip always includes a mass of dense protoplasm with a number of nuclei. The nuclei are said to fragment in the conidia, dividing by constriction in the middle. On germination the conidium puts forth a tube, and the multinucleate protoplasm streams forward into it. The conidia of *Entomophthora Delpiniana* are uninucleate, perhaps for the reason that the conidiophores show a distinct tendency to pass from the condition of a cœnocyte to that of a segmented branched filament. The greater portion of the contents of the terminal cells, with a single nucleus, passes into a bud-like process which becomes cut off below by constriction to develop the conidium.—B. M. DAVIS.

IN a recent number of *Science*,[13] Professor C. S. Slichter of the University of Wisconsin proposes to use the kinetoscope for the reproduction and magnification of slow motions such as the growth, development, and movements of plants, flow of plastic substances, etc. His suggestion seems so likely to prove fruitful that we reproduce part of his note.

> The method that I selected . . . . was as follows: Let the moving body be photographed upon kinetoscope film at stated intervals — every few minutes, or every few hours, as the case may require. After a sufficient number of these photographs have been obtained, the film may be run through an ordinary projecting kinetoscope at the usual rate. In this way the motion that has required several weeks for its production may be reproduced upon the screen within the limits of a few minutes or seconds. I have magnified in this way the rate of motion about 500,000 fold, but of course there is no major limit to the possible rate of magnification. I made the first application of this method of magnifying slow motions to the motion of growing seedlings. Several peas and beans were placed in a glass root cage containing wet sand. The photographs were taken by artificial light at fixed intervals day and night for about three weeks. When the film is run through the kinetoscope the entire growth for the period of three weeks is reproduced in a few seconds. . . . . The kinetoscope also shows very clearly the different speeds at which the various parts of the plant grow,

[12] CAVARA: Osservazioni citologiche sulle Entomophthoreæ. Nuovo Giorn. Bot. Ital. **6**: 411. 1899.

[13] Science **11**: 535. 6 Ap. 1900.

and the different speeds at which the same part grows at different times. The greatest variety in the rate of growth exists, as I suppose is well known, and of course the kinetoscope brings out the relative rates of growth in a very truthful and graphic manner. . . . . At the present time I am preparing some additional films taken from growing seeds. Of course there is no reason why the photographing should not be continued until the plants have bloomed and fruited, if any fact important to mechanics or botany is likely to result from the trouble. Perhaps botanists know of matters in plant growth and plant development that it may pay them to investigate by the same method. I anticipate that some interesting facts concerning the mechanics of the root's motion into and through the soil will result from such studies.

MR. ALBERT F. WOODS, of the U. S. Division of Vegetable Physiology and Pathology, has found that chlorophyll is rapidly destroyed by oxidizing enzymes, which are normally present in small quantity in many of the higher plants. Under unknown conditions these enzymes become more active or are produced in larger quantity, causing disease marked by variegation, such as the mosaic disease of tobacco, which may be produced at will by inoculation with germ-free virus from diseased areas, and by cutting back healthy pot-grown plants which have about exhausted the soil in the pot and allowing only one shoot to develop rapidly in a high temperature under copious watering. The lighter areas invariably show the presence of larger amounts of some substance which turns a solution of gum guaiac blue. This reaction Mr. Woods accepts as indicating the presence of oxidizing enzymes, though aware that it has been considered unreliable. The oxidase and peroxidase may remain in soil uninjured for several months. The latter diffuses readily in plant tissues or agar plates, and may be dried without injury.[14]

In a short note in *Science* **11**: 17–19. 1900, Mr. Woods presents reasons for believing that in the mosaic disease of tobacco leaves the lighter colored areas contain more starch than the healthy tissues because the greater amounts of oxidase they contain partly or wholly inhibits the action of translocation diastase.—C. R. B.

PROFESSOR D. H. CAMPBELL has published some of the results of his studies of Araceæ in the current *Annals of Botany* (**14**: 1–25. *pls. 3*. 1900). Species of Dieffenbachia and Aglaonema were the forms from which he obtained the most complete results, although very many gaps remain to be filled even in them. In both of these cases the axial origin of the ovule seems undoubted, and this probably represents a primitive condition. One of the most striking facts presented by these forms, as well as others of the family, is the early development of a compact endosperm which completely fills the sac, and in some cases is probably developed before fertilization, and certainly often appears when no embryo is formed. In the whole family there is also a tendency for the antipodal cells to develop strongly, often dividing

[14] Centralbl. f. Bakt. 5^2: 745–754. 1899.

and forming a tissue which supplements the endosperm. The embryo is also somewhat peculiar and suggestive. The failure to develop a suspensor is thought by Professor Campbell to be associated with the complete investment of the embryo by the endosperm. Two types of segmentation of the young embryo which were noted are: (1) the formation of two transverse divisions before any longitudinal walls appear, and (2) a regular quadrant formation suggestive of the first divisions of the fern embryo. The cotyledon is exceedingly large as compared with the stem and root, the last organ appearing late and being rather lateral in origin, as in Lysichiton and Pistia.

One of the peculiarities of Dieffenbachia is that the single archesporial cell divides transversely very unequally, the outer cell being the larger and becoming the embryo sac. The inner cell may divide once or not at all. The embryo sac in its development encroaches upon the whole of the nucellar tissue and finally is in contact with the inner integument. A nucellar cap, however, remains at the micropylar end of the sac, and its cells enlarge and divide, forming a somewhat permanent tissue, and suggesting the condition of things which Smith has found in Pontederiaceæ and Merrell in Silphium.

The characteristics of the group which have been developed so far, and which the author regards as primitive, are the axial ovule, the early development of a solid endosperm, the great development of the antipodal cells, and the absence of a suspensor.—J. M. C.

A NEW GENUS of the Volvocaceæ from the streams of central Illinois, has been described by Kofoid[15] and named *Platydorina*. It forms a flat horseshoe-shaped colony of sixteen or thirty-two cells, the plate being somewhat twisted into a left spiral. The rounded side of the outline is the anterior end, and the portion corresponding to the heel of the horseshoe is prolonged into three or five tails, hence the name of the single species, *P. caudata*. The contents of the cells are bright green, and each has a pair of cilia and generally the red pigment spot. The tails are formed of intercellular gelatinous matter, and they, with the peculiar twist of the colony, present the most prominent specific characters. No method of sexual reproduction has been discovered.

The asexual reproduction is by the repeated division of gonidial cells in the whole or part of the parent colony. The early stages of this segmentation are identical with those of Eudorina and Pleodorina. The colony becomes cup-shaped, then ellipsoidal, but finally flattens so that the cells from two sides are pressed together. They become intercalated in a common plate, in such a regular manner that every alternate cell has the cilia and pigment spot uppermost to the observer, while the other cells present the

[15] KOFOID: On Platydorina, a new genus of the family Volvocidæ, from the plankton of the Illinois river. Bull. Ill. State Lab. of Nat. Hist. 5, 1899.

view with the pyrenoid most prominent. From this method of development it would appear that Platydorina is not a more specialized Gonium, but derived from ancestors of the Eudorina type. Platydorina is positively phototactic, exhibiting this habit to a conspicuous degree, but avoiding bright sunlight. The movement is always forward unless obstructions are met, and the rotation as a rule is from right over to left. This is the direction naturally favored by the resistance that the water offers to a moving body twisted in the manner of this coenobium. However, as the rotation in Pleodorina and Eudorina is predominately from right over to left, it is possible that the form of the colony in Platydorina is the result and not the cause of this rotation. Dr. Kofoid discusses the use of the term colony, as unfortunately inappropriate to the higher members of the Volvocaceæ, where there is a certain degree of cell differentiation, especially in Pleodorina and Carter's form of Eudorina, with the probability of a physiological cooperation between the cells of Volvox. Which facts make this group of plants a most interesting one from a speculative and evolutionary standpoint, and perhaps we may pardon their failure to accommodate themselves to a system that makes possible the use of exact terms.—B. M. DAVIS.

NEWS.

PROFESSOR O. MATTIROLO, of Florence, has been appointed professor of botany in the University of Turin.

DR. HUGO ZUKAL, associate professor of botany in the Agricultural Institute at Vienna, died on February 15, at the age of 55.

PROFESSOR A. N. BERLESE, of Bologna, has been appointed assistant professor of botany and director of the botanical institute, at Sassari, in Sardinia.

DR. EDWIN B. ULINE has been appointed special assistant in the Gray Herbarium for the summer, during the absence of Dr. B. L. Robinson in Europe.

PROFESSOR M. A. BARBER, associate professor of botany in the University of Kansas, will spend six months in Berlin in bacteriological study. He will resume his university duties in the autumn.

THE MARINE BIOLOGICAL LABORATORY offers this summer for the first time a course in nature study. It is to occupy the entire session of six weeks, and will be conducted cooperatively by members of the staff of the laboratory and a number of specialists. The thoroughness characteristic of the work of this institution is thus assured. We note among those outside of the staff the names of Wheeler, Dudley, and Peckham in entomology; Chapman, Herrick, Stone, and Dearborn on birds; Hodge on the toad; Patten on the king crab; Scott and Penhallow in paleontology; and McFarlane in phanerogamic botany. It is quite safe to say that never before in this country has a course in nature study been presented by a group of men so strong in their respective lines. The time will be divided as follows: cryptogamic botany one week, phanerogamic botany one week, the king crab two days, insects four days, birds one week, the toad one day, marine invertebrates one week, animal psychology six days. A few sentences from the introduction to the program, written by Dr. Whitman, shows the temper of the course: "We shall have field work, laboratory exercises, lectures, demonstrations, and all available means of reading a few chapters in nature's book. Study nature for understanding, not for information, is the ideal to be kept in view. Those who are ambitious to fill their notebooks with a complete survey of the field of facts are advised not to apply. We shall be content if we succeed in taking a few soundings at advantageous points." Announcement of the course may be obtained from Dr. Bradley M. Davis, University of Chicago.

AFTER OCCUPYING for twelve years small and in many respects unsuitable rooms in the old college buildings, the botanical department of University College, Liverpool, is at length to be housed in a new and commodious institute, the munificent gift of Mr. W. P. Hartley, of Aintree, Liverpool. The building is 37 by 85 feet, and five stories high. The museum will con-

HARTLEY BOTANICAL INSTITUTE

tain not only morphological specimens illustrative of the scientific aspect of botany, but also specimens of all products of the vegetable kingdom used in the arts, such as timbers, pharmaceutical products, cottons, hemp, flax, food-products, both in the raw and in the manufactured state. There is also provided an ample equipment of lecture halls, herbarium rooms, elementary laboratories, and also laboratories for research in morphology and physiology. Mr. Hartley's gift will provide University College, Liverpool, with a botanical laboratory worthy to stand alongside the pathological and physiological laboratories, the recent gift of Mr. Thompson Yates to the college. The building will in all probability be ready for occupation before the beginning of the autumn of 1901.